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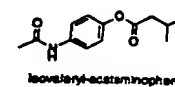
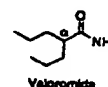
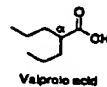
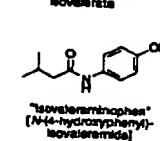
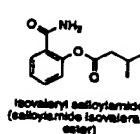
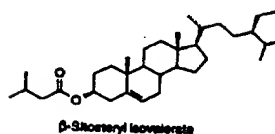
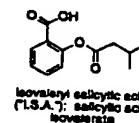
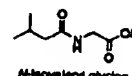
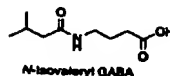
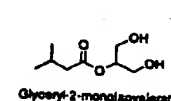
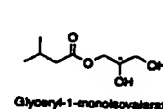
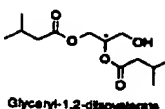
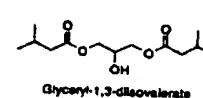
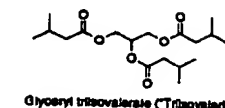
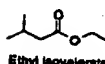
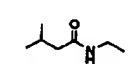
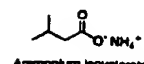
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(54) Title: TREATMENT OF SPASTICITY, CONVULSIONS BY ISOVALERIC ACID DERIVATIVES CNS DEPRESSANTS

(57) Abstract

Preparations and extracts of valerian, as well as isovaleramide, isovaleric acid, and its pharmaceutically acceptable salts, esters, and substituted amides, exhibit clinically significant pharmacological properties which implicate a treatment for a variety of pathological conditions, including spasticity and convulsions, which are ameliorated by effecting a mild depression of CNS activity. The compositions in question generally are non-cytotoxic and do not elicit weakness or sedative activity at doses that are effective for the symptomatic treatment of such pathological conditions.



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TREATMENT OF SPASTICITY, CONVULSIONS BY ISOVALERIC ACID DERIVATIVES CNS
DEPRESSANTS**BACKGROUND OF THE INVENTION**

5 The present invention relates to treating pathological conditions, such as spasticity and convulsions, the symptoms of which are alleviated by a mild depression of activity in the central nervous system (CNS), without producing undesirable excessive sedation or muscle weakness in animal subjects, including humans. More particularly, the invention relates to the therapeutic use of isovaleramide, isovaleric acid, and related compounds in patients suffering from pathologies of this nature.

10 Many agents currently employed in the treatment of pathologies such as spasticity and convulsions display troubling side-effect profiles which limit their long-term clinical utility. Among these agents, for example, 15 are the benzodiazepines, which can cause cognitive blunting. Two other agents are valproate, which exhibits hepatotoxicity, and baclofen, which can produce excessive muscle weakness and sedation, limiting the therapeutic potential for both drugs.

SUMMARY OF THE INVENTION

20 Accordingly, it is an object of the present invention to provide a therapeutic approach for the treatment of various pathologies by effecting a mild depression in CNS activity without producing excessive sedation, muscle 25 weakness, fatigue, or hepatotoxicity.

It also is an object of the present invention to provide a method for alleviating one or more symptoms associated with a condition, such as spasticity, that is ameliorated by means of a centrally mediated decrease in muscle tone.

It is another object of the present invention to provide a novel anticonvulsant therapy.

In accomplishing these and other objectives, there has been provided, according to one aspect of the present invention, a use of a compound selected from the group consisting of isovaleric acid, a pharmaceutically acceptable salt of isovaleric acid, a pharmaceutically acceptable ester of isovaleric acid, and a pharmaceutically acceptable amide of isovaleric acid in the preparation of a pharmaceutical formulation for use in a method of treating a pathology that is ameliorated by a mild depression of CNS activity, whereby at least one symptom of said pathology is alleviated. Thus, the present invention also contemplates a treatment method comprising the step of administering, to a patient suffering from a pathology that is ameliorated by a mild depression of CNS activity, a therapeutically effective amount of a pharmaceutical formulation comprising a pharmaceutically acceptable carrier and a composition selected from the aforementioned group of agents.

Pursuant to one embodiment of the invention, the treated pathology is an affective mood disorder, convulsions, a central neuropathic pain syndrome, a headache, or a restlessness syndrome. For another embodiment, the pathology in question is ameliorated by a centrally mediated decrease in muscle tone, and is illustrated by spasticity.

In accordance with another aspect of the present invention, a use is provided for an extract of Valerianaceae, cramp bark, black haw, or hops in the preparation of a pharmaceutical formulation for use in a

method of treating a symptom of spasticity, where the extract comprises at least one compound that is hydrolyzed *in vivo* to yield isovaleric acid or isovaleramide. By the same token, the present invention provides a method for alleviating a symptom of spasticity in a subject in need of such treatment, comprising the step of administering a therapeutically effective amount of an extract as described above.

Other objects, features, and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating preferred embodiments of the present invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts the structures of various compounds, including isovaleramide.

Figure 2 portrays the effect of isovaleramide (at 300 mg/kg, *i.p.*) on gross observational spasticity scores elicited by a metal probe applied to the abdomen in the chronic spinalized rat. Each rat served as its own control; there were three rats per group. The bar at time zero represents pre-treatment control values.

Figure 3 illustrates a dose- and time-dependent reduction of the flexor reflex, an electrophysiological measure of spasticity, in the chronic spinalized rat. The effects of isovaleramide (300, 600, and 1200 mg/kg *p.o.*), baclofen (10 mg/kg *s.c.*), and vehicle (water, 12 ml/kg *p.o.*) are shown at pre-treatment (time zero) and at 30, 60, 90, and 120 minutes post-administration. At all

5 doses, isovaleramide caused a significant decrease in the magnitude of the flexor reflex, comparable to that observed with baclofen. Statistical significance was assessed by one-way analysis of variance (ANOVA) and post-hoc Dunnett's t-test: $p < 0.05$ (*); $p < 0.01$ (**); NS = not significant.

10 Figure 4 shows that isovaleramide and baclofen, a known antispasticity agent, produced a similar reduction of the flexor reflex in the chronic spinalized rat. The responses from Figure 3 were converted to a total-area-under-the-curve for the two-hour measurement. All drug-related groups were significantly different from the vehicle ($p < 0.05$, ANOVA).

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

15 1. OVERVIEW

20 The inventors have discovered that isovaleric acid and its pharmaceutically acceptable salts, amides such as isovaleramide, and alcohol esters such as ethyl isovalerate and β -sitosteryl isovalerate can be administered in vivo to effect a mild depression of CNS activity. That is, these agents depress CNS activity, by enhancing inhibitory (or decreasing excitatory) neurotransmission centrally, without complete suppression of all activity. Pursuant to the present invention, therefore, a subject who receives such an agent is not overtly sedated, anesthetized, or paralyzed in the context, for example, of decreasing seizures (no anesthesia), decreasing muscle tone (no paralysis), eliciting a calmative effect (no sedation), or ameliorating an ambulatory syndrome such as spasticity (no weakness or flaccidity).

30 A number of pathologies, exemplified by affective mood disorders, headaches (chronic, cluster, migraine),

restlessness syndromes, neuropathic pain, movement disorders, spasticity, and convulsions, have at least one symptom that is alleviated by a mild CNS depression. Accordingly, an individual who suffers from such a pathology is a candidate for therapy that entails, pursuant to the present invention, the individual's receiving a pharmaceutical formulation of isovaleramide, isovaleric acid, or a related compound.

It is believed that the compounds of the present invention act via a GABAergic mechanism and, hence, bear a pharmacological similarity to known drugs which are considered to enhance central GABAergic neurotransmission. Like many of the extant drugs, such as the barbiturates, the benzodiazepines, gabapentin, valproate, vigabatrin, and progabide, the compounds of the present invention are effective in treating pathological conditions, illustrated by those mentioned above, that are thought to arise from a defect in the regulation of inhibitory (GABA- and/or glycine-related) neurotransmission. This regulation may occur by a direct or modulatory effect at CNS receptors or by impact on a metabolic pathway which heightens GABA or glycine levels and/or which reduces levels of an excitatory neurotransmitter like glutamate. See Ruggero et al., in ANTIEPILEPTIC DRUGS (4th ed.), pages 581-88 (Raven Press 1995); Nogrady, MEDICINAL CHEMISTRY: A BIOCHEMICAL APPROACH (2d ed.), pages 225-39 (Oxford University Press 1988); Fonnum and Morselli, respectively, in PSYCHOPHARMACOLOGY: THE THIRD GENERATION OF PROGRESS, pages 173-82 and 183-95 (Raven Press 1987). Despite a pharmacological analogy to the known drugs mentioned above, however, the present invention surprisingly does not engender the disadvantageous side effects associated with conventional drug therapies in this area, such as the hepatotoxicity that arises with valproate administration.

2. EXEMPLARY PATHOLOGIES AMELIORATED BY A MILD DEPRESSION OF CNS ACTIVITY

SPASTICITY: "Spasticity is frequently defined as an upper [i.e., CNS] motor neuron disorder characterized by a velocity dependent increase in tonic stretch reflexes (muscle tone) with exaggerated tendon jerks resulting from hyperexcitability of the stretch reflex." Lance, Symposia synopsis in SPASTICITY - DISORDERED MOTOR CONTROL, Feldman et al. (eds.) (1980). An increase in tonic stretch reflexes, however, is only one of the many symptoms present in a disordered motor function caused by an upper neuron lesion in a variety of neurological disorders; thus, such a disordered motor function is variable in its etiology and presentation.

Major disease states and conditions associated with spasticity include multiple sclerosis, cerebral palsy, stroke, trauma or injury to the spinal cord, and closed head trauma. There are "positive symptoms" that can occur with spasticity, such as the Babinski response, painful flexor or extensor spasms, increased or exaggerated deep tendon reflexes, and clonus. Other symptoms, referred to as "negative symptoms," include weakness, fatigue, lack of dexterity, and paralysis. It is the combination of these positive and negative signs and symptoms that is denoted clinically as "spastic paresis" (spastic paralysis). Pain, impairment of sleep, and various degrees of loss of general motor function are also associated with spasticity.

The pathological states observed in spasticity are fundamentally different at the physiological level from the commonly experienced acute muscular aches, strains, and sprains that occur from a localized external insult to a particular muscle, i.e., outside of or peripheral to the CNS. These pathological states also are different from the relatively common involuntary spasms of smooth muscle, such as vascular spasms, bladder spasms, and

5 bronchial spasms. Such non-spastic (non-CNS), peripheral or localized symptoms are commonly treated with so-called "antispasmodic" or "spasmolytic" agents, but these generally are not useful in treating spasticity. Cedarbaum & Schleifer, "Drugs for Parkinson's Disease, Spasticity and Acute Muscle Spasms," in GOODMAN AND GILMAN'S THE PHARMACOLOGICAL BASIS OF THERAPEUTICS, 8th ed. [hereafter GOODMAN AND GILMAN'S], pages 463-484 (Pergamon Press 1990).

10 The pharmaceutical formulations employed in accordance with the present invention can effect a centrally mediated decrease in muscle tone and, hence, are useful for the acute or chronic alleviation of one or more symptoms of spasticity. In this context,
15 "spasticity" refers to a heightened tone of skeletal muscle which is manifested by symptoms such as (but not limited to) painful flexor or extensor spasms, increased or exaggerated deep tendon reflexes, hyperreflexia, loss of dexterity, muscular weakness, exaggerated tendon jerks, and clonus. The phrase "antispasticity agent" refers here to a composition that is useful for the symptomatic treatment of spasticity, as demonstrated by the alleviation of at least one of the following
20 manifestations of spasticity: painful flexor or extensor spasms, increased or exaggerated deep tendon reflexes, hyperreflexia, loss of dexterity, muscular weakness, exaggerated tendon jerks, and clonus. Accordingly, the "alleviation" of spasticity refers here to the lessening of one or more symptoms of spasticity, including but not limited to painful flexor or extensor spasms, increased or exaggerated deep tendon reflexes, hyperreflexia, loss of dexterity, muscle weakness, exaggerated tendon jerks, and clonus.

30 Spasticity is associated with multiple sclerosis, stroke, head trauma, spinal cord injuries, cerebral palsy, and other neurodegenerative diseases, disorders,
35

and conditions. Spasticity is distinct from acute muscle spasms, which may be associated with a variety of conditions different from those leading to spasticity. These acute muscle spasm-causing conditions include trauma, inflammation, anxiety, and/or pain.

The difference between spasticity and acute muscle spasms is illustrated by the fact that agents useful for the treatment of muscle spasms are not useful for treating spasticity associated with chronic neurological diseases. Cedarbaum & Schleifer (1990), *supra*. Likewise, agents used heretofore to treat spasticity associated with chronic neurological disorders have not been employed in treating acute muscle spasms, except for the benzodiazepines, such as diazepam (Valium®), which are recognized also to have muscle-relaxant activity as well as anxiolytic and analgesic properties. By contrast, the present invention achieves a centrally mediated decrease in muscle tone which, in turn, addresses the particular symptoms of spasticity.

CONVULSIVE DISORDERS: Due to the widespread availability of reasonably predictive and experimentally accessible animal models of convulsant states, a number of clinically useful anticonvulsants have been prepared and developed. For example, see Cereghino et al., "Introduction," in *ANTIEPILEPTIC DRUGS*, 4th ed., pages 1-11 (Raven Press 1995). "In many patients, seizures can be controlled with currently available antiepileptic drugs, but 25 to 30 percent of patients continue to have seizures despite optimal therapy, while many others experience unacceptable side effects." Dichter et al., *Drug Therapy* 334: 1583 (1996).

Thus, many anticonvulsants in clinical use are plagued by the occurrence of significant side effects, including troublesome daytime sedation, muscular weakness, tolerance, gingival hyperplasia, blood dyscrasias, and potentially fatal hepatotoxicity. Many

of these side effects are especially of concern in the clinical management (treatment) of epilepsy in children.

5 The present invention can be used to treat convulsive disorders such as epilepsy. That is, the pharmaceutical compositions of the invention display "anticonvulsant activity," which is evidenced by a reduction of the severity, number, or duration of convulsions in animal models of epilepsy. Accordingly, the inventive pharmaceutical compositions should be useful in treating
10 conditions such as but not limited to simple partial seizures, complex partial seizures, status epilepticus, and trauma-induced seizures, as occur following head injury or surgery.

15 Epilepsy is a common disorder which has many causes, and it can be very difficult to control, often requiring treatment for many years to keep seizures under control. "At this time, there is no satisfactory treatment for epilepsy in a substantial proportion of patients. Clinical trials have shown that certain patients have a
20 better response to one drug than another, even when the patients have similar types of seizures and the drugs have similar mechanisms of action. The frequency and severity of side effects also varies substantially. Thus, multiple medications with different mechanisms of
25 action and attendant side effects will be needed for treatment of epilepsy until either epilepsy can be cured or a potent, safe new drug with broad activity is discovered" and developed. Dichter et al. (1996), *supra*.

30 **AFFECTIVE MOOD DISORDERS:** Under this rubric are conditions ranging from depression to dysphoric mania, i.e., mania, schizoaffective disorder, traumatic brain injury-induced aggression, post-traumatic stress disorder, panic states, and behavioral dyscontrol
35 syndromes. Affective mood disorders have been treated primarily prophylactically, with lithium salts, since the 1950s in Europe and since the 1970s in the United States.

Emrich et al., *J. Affective Disorders* 8: 243-50 (1985). In recent years, alternatives to lithium treatment have been under development, given several problems with lithium therapy. Newer, alternative therapies to lithium for affective mood disorders are therapies with anticonvulsants such as carbamazepine, benzodiazepines, valpromide, and valproate. Bernasconi et al., in *ANTICONVULSANTS IN AFFECTIVE DISORDERS*, pages 14-32 (Excerpta Medica 1984). Valproate has a lower propensity towards depressed arousal and mentation, memory impairment, and cognitive blunting than is seen with the benzodiazepines.

Despite the demonstrated efficacy of valproate in a multitude of affective disorders, the hepatotoxicity, mutagenicity, and gastric upset observed with its administration highlights the need for new therapeutic agents and treatments with improved side effect profiles. A pharmaceutical formulation according to the present invention is effective to this end, especially with respect to improved side effects. Notwithstanding any perceived structural similarity between, for example, valpromide and isovaleramide (see Figure 1), an absence is expected of valproate-related side effects which otherwise might detract from the efficacy of the present invention in treating the range of affective mood disorders.

CENTRAL NEUROPATHIC PAIN SYNDROMES: Conditions in this category, involving "neuropathic pain," affect a significant number of patients suffering from disorders of the brain or spinal cord, such as stroke, trauma, multiple sclerosis, and diabetes. Casey, in *PAIN AND CENTRAL NERVOUS SYSTEM DISEASE* (Raven 1991). Many GABAergic compounds are efficacious in various analgesia models relevant to identifying therapeutic candidates for treating neuropathic pain. See Lloyd & Morselli, in *PSYCHOPHARMACOLOGY: THE THIRD GENERATION OF PROGRESS*

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(Raven Press 1987). In a related vein, the use of anticonvulsants like valproate to treat various pain states has been documented extensively. Swendlow, J. *Clin. Neuropharmacol.* 7: 51-82 (1984). Thus, a pharmaceutical formulation of the present invention can be applied in similar fashion to ameliorate neuropathic pain.

HEADACHES: Headaches of the migraine type (Hering & Kuritzky, *Cephalalgia* 12: 81-84 (1992)), the cluster type (Hering & Kuritzky, *loc. cit.* 9: 195-98 (1989)) and the chronic type (Mathew & Sabiha, *Headache* 31: 71-74 (1991)) have been treated by the administration of valproate. Interactions with the GABAergic system are thought to play a major role in the etiology of these headaches and in the associated valproate therapy. For this reason, the present invention can alleviate symptoms associated with each of the three headache types, without the adverse side effects of valproate therapy.

RESTLESSNESS SYNDROME: The phrase "restlessness syndrome" denotes a somatic (non-mental) restlessness characterized by involuntary movement of the limbs, as well as by a sense of physical (rather than mental) agitation, which is independent of mood and, hence, is distinguished from restlessness *per se*. See Sachdev et al., *Austral. New Zealand J. Psychiatry* 30: 38-53 (1996).

The genus of restlessness syndromes, inclusive of numerous indications, can be observed in association with many organic and non-organic psychiatric illnesses. For example, drug-induced restlessness (tardive, chronic, and withdrawal akathisia), such as drug-induced extrapyramidal symptoms, is one of the most common side effects of neuroleptic drug therapy. Also within the restlessness-syndrome rubric are the so-called "restless leg syndrome" and "sleep-related periodic leg movements," pathologies that can be associated with head and/or spinal cord trauma and with lesions of the spinal cord.

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Idiopathic restless leg syndrome follows an autosomal dominant inheritance, with a variable clinical expression of symptoms.

Diminished GABAergic neurotransmission is implicated in the neurochemical basis of restlessness syndromes. Consistent with this notion, for instance, is the efficacy of the benzodiazepines, baclofen, valproate, and gabapentin in the treatment of restless leg syndrome, an important indication. See O'Keefe, *Arch. Intern. Med.* 156: 243-48 (1996); Danek et al., in *NEUROLOGICAL DISORDERS: COURSE AND TREATMENT*, pages 819-23 (Academic Press 1996); Mellick & Mellick, *Neurology* 45(suppl): 285-86 (1995). More generally, the present invention provides an effective therapy for restlessness syndromes with minimal side effects.

MOVEMENT DISORDERS: Various GABAergic agents are known to decrease the dyskinetic movement characterizing movement disorders such as Parkinson's disease, Huntington's chorea, tardive dyskinesia, and stiff-man syndrome. This fact has highlighted a role for central GABAergic function in the control and modulation of CNS excitability and movement. Lloyd & Morselli (1987), *supra*. By the same token, a therapy within the present invention can effect a reduced level of CNS activity, presumably via a GABAergic mechanism, to alleviate one or more symptoms of a movement disorder.

3. METHODS FOR PREPARING PHARMACEUTICAL FORMULATIONS

The rhizomes and roots of *Valeriana* spp. (common name: valerian; family Valerianaceae) have been used for medicinal purposes since ancient times. The most commonly used valerian preparations include aqueous and hydroalcoholic extracts, such as tinctures, intended for oral administration. In addition, ammoniated valerian tinctures were used medicinally in the English-speaking world since at least the beginning of the seventeenth

century. Hobbs, *HerbalGram* No. 21: 19-34 (1989). In the last three decades, the sedative and antispasmodic properties of valerian preparations have been attributed primarily to the presence of chemically labile monoterpenoid iridoid triester compounds called valepotriates ("valerian-epoxy-triesters (-ates)").

The most common and abundant of the valepotriates, valtrate and didrovaltrate, each contain two isovalerate moieties esterified to a "central" iridoid nucleus. Lin et al., *Pharm. Res.* 8: 1094-02 (1991). These acid- and heat-labile substances do not survive intact in the stomach following oral administration, however, readily releasing two moles of isovaleric acid for every mole of valepotriate. Furthermore, aqueous extracts of valerian rhizomes and roots retain their biological properties, even though the valepotriate triesters are water-insoluble. Bos et al., *Phytochem. Anal.* 7: 143-51 (1996).

The major, water-soluble, active principle of commonly used valerian extracts and other preparations, such as aqueous or hydroalcoholic extracts or tinctures, has been determined to be the ester hydrolysis product, isovaleric acid. Ammonium isovalerate and isovaleramide are produced in ammoniated tinctures. Balandrin et al., *J. Toxicol.-Toxin Rev.* 14: 165 (1995). The structures of isovaleramide and related compounds are depicted in Figure 1. In this way, the chemically labile valepotriates and other valerian-derived monoterpenoid-isovalerate esters, such as bornyl, lavandulyl, and ethyl isovalerates, act as "pro-drugs" and chemical precursors for isovaleric acid, its salts, and isovaleramide.

Isovaleramide has been isolated from valerian plants, most probably as an isolation artifact following treatment with ammonia. Buckova et al., *Cesk. Farm.* 26: 308 (1977); *Chem. Abstr.* 88: 86063z (1978); see also Bos et al. and Fuzzati et al., *Phytochem. Anal.* 7: 143, 76

(1996). More Recently, isovaleramide was shown to exhibit low acute toxicity *in vivo*, no mutagenic potential, and clinically useful anxiolytic properties. U.S. patent No. 5,506,268; PCT application WO 94/28,888. Methods for preparing isovaleramide are well known.

Extracts of medicinal plants that are useful for treating the symptoms of spasticity and convulsions can be prepared by aqueous, hydroalcoholic, or alcoholic extraction, or by extraction with other suitable solvents using methods well known to those of skill in the art. In the context of the present invention, useful extracts contain at least one of the following: isovaleric acid, its salts or complexes, ethyl isovalerate, isovaleramide, N-ethyl isovaleramide, and their chemical precursors. Useful extracts also share the common property of releasing isovaleric acid and/or isovaleramide upon hydrolysis *in vivo*. Standard methods for preparing such extracts can be found in pre-1950 editions of the U.S. PHARMACOPOEIA (U.S.P.) and the NATIONAL FORMULARY (N.F.), as well as in well-known references such as Gennaro (Ed.), REMINGTON'S PHARMACEUTICAL SCIENCES, 18th ed. (Mack Publishing Co. 1990), Tyler et al., PHARMACOGNOSY, 9th ed. (Lea and Febiger 1988), and Hare et al., THE NATIONAL STANDARD DISPENSATORY (Lea Brothers 1905). Additional citations appear in U.S. patent No. 5,506,268 and PCT application WO 94/28,888.

The principal historic sources of naturally occurring isovaleric acid have been valerian rhizomes and roots, as well as those of closely related plants in the family Valerianaceae. As discussed by Hobbs (1989), *supra*, these include the common valerian plant, *Valeriana officinalis* L., as well as the East Indian valerian, *V. wallichii* DC. and the biblical spikenard, *Nardostachys jatamansi* (Roxb.) DC. In addition to valerian rhizomes and roots, other plants which have been used traditionally as sedative or "antispasmodic" herbal

medicines are known to contain, or to produce, isovaleric acid. These include hops (*Humulus lupulus* L., family Moraceae, which is often used in herbal formulations in combination with valerian), "cramp bark" or "guelder rose" (*Viburnum opulus* L., family Caprifoliaceae), and "black haw" (*V. prunifolium* L., root bark). Hare et al., THE NATIONAL STANDARD DISPENSATORY, pages 93, 94, 159, 160, 169, 256, 642, 692-694, 766, 767, 932, 1031, 1383, 1384, 1426, 1479, 1480, 1571, 1572, 1619, 1620, 1631-1633, 1661, and 1662 (Lea Brothers 1905); Heyl et al., J. Am. Chem. Soc. 42: 1744 (1920); Grier, Pharm. J. Pharm. 68: 302 (1929); Grier, Chem. Drug. (London) 110: 420 (1929); Grieve, A MODERN HERBAL, pages 35-40, 265-276, 381, 382, 411-415, 744-746, 781, 782, and 824-830 (Hafner 1959); Holbert, J. Am. Pharm. Assoc., Sci. Ed. 35: 315 (1946); Hoffmann, THE HERBAL HANDBOOK: A USER'S GUIDE TO MEDICAL HERBALISM, pages 38, 39, 83 and 84 (Healing Arts Press 1989).

As in the case of valerian rhizomes and roots, hops generate isovaleric acid from more chemically complex precursors upon oxidation or enzymatic breakdown. Millspaugh, AMERICAN MEDICINAL PLANTS, AN ILLUSTRATED AND DESCRIPTIVE GUIDE TO THE AMERICAN PLANTS USED AS HOMEOPATHIC REMEDIES, pages 622-626 (Dover 1974); Hare et al., THE NATIONAL STANDARD DISPENSATORY, pages 766-767 (Lea Brothers 1905); Grier, Chem. Drug. (London) 110: 420 (1929); Grieve, A MODERN HERBAL, pages 411-415 (Hafner 1959); Stevens, Chem. Rev. 67: 19 (1967); Duke, CRC HANDBOOK OF MEDICINAL HERBS, page 557 (CRC Press 1985).

Pharmaceutically acceptable salts of organic acids, such as isovaleric acid, which have been approved by the U.S. Food and Drug Administration for commercial marketing include sodium, potassium, lithium, zinc, aluminum, calcium, or magnesium salts. REMINGTON'S PHARMACEUTICAL SCIENCES, 18th ed., page 1445 (Mack

Publishing Co. 1990). Salts of isovaleric acid that are commercially available in the United States include the ammonium, sodium, potassium, and zinc isovalerates.

Pharmaceutically acceptable alcohols can form esters with isovaleric acid via the corresponding isovaleric acid chloride and/or anhydride by methods that are well known in the art. See, for example, March, ADVANCED ORGANIC CHEMISTRY: REACTIONS, MECHANISMS, AND STRUCTURE, fourth ed. (John Wiley and Sons 1992). Such alcohols contain at least one hydroxyl or phenol moiety, and are well tolerated *in vivo*. Examples of suitable alcohols include ethanol, certain carbohydrates and related compounds such as glucose, fructose, sucrose, xylose, and lactose, sugar alcohols such as dulcitol, mannitol, and sorbitol, sugar acids such as gluconic and glucuronic acids, glycerol, the polyol inositol, benzyl alcohol, certain phenols such as phenol, salicylic acid, saligenin, salicylamide, vanillin, *p*-hydroxycinnamic acid (*p*-coumaric acid), caffeic acid, ferulic acid, gallic acid, ellagic acid, quercetin, and eugenol. Other examples of suitable alcohols include alkaloids and biogenic amines such as ephedrine, pseudoephedrine, phenylpropanolamine, tyramine, and dopamine, vitamins such as ascorbic acid (vitamin C), thiamine (vitamin B1), riboflavin (vitamin B2), pyridoxine (vitamin B6), cyanocobalamin (vitamin B12), the tocopherols (vitamin E), choline, folic acid, and pantothenic acid, monoterpene alcohols such as geraniol, nerol, and linalool, naturally occurring triterpene alcohols such as α - and β -amyrins, lupeol, and oleanolic and ursolic acids, bile acids such as cholic acid, deoxycholic acid, and taurocholic acid, and common naturally occurring plant sterols (phytosterols) such as β -sitosterol, stigmasterol, campesterol, and brassicasterol. Tyler et al., PHARMACOGNOSY, 9th ed. (Lea and Febiger 1988). Other such well-tolerated hydroxyl- and phenol-containing

compounds can be readily identified by those skilled in the art by consulting standard reference works such as THE MERCK INDEX and REMINGTON'S PHARMACEUTICAL SCIENCES, 18th ed. (Mack Publishing Co. 1990). Esters of isovaleric acid that are commercially available in the United States include the bornyl, ethyl, n-butyl, isoamyl, and geranyl isovalerates.

Isovaleric acid, ammonium isovalerate, and the esters ethyl isovalerate, isoamyl isovalerate, 2-methylbutyl isovalerate, cinnamyl isovalerate, methyl isovalerate, bornyl isovalerate, isobornyl isovalerate, and menthyl isovalerate, among other isovalerate esters, are listed in the Code of Federal Regulations by the FDA as being acceptable flavoring agents which may be used in foods. 21 CFR §172.515 (1991). Valerian (*Valeriana officinalis* L.) rhizomes and roots and black haw (*Viburnum prunifolium* L.) bark are listed as acceptable natural flavoring substances and natural adjuvants in 21 CFR §172.510 (1991). Hops and "lupulin" are listed among substances that are generally considered to be safe ("GRAS"). 21 CFR §182.20 (1991).

Generally, esters of isovaleric acid are expected to be hydrolyzed *in vivo* by ubiquitous esterase enzymes, thereby releasing isovaleric acid and the constituent alcohol or phenol. Particularly preferred among the isovalerate esters are glyceryl mono-, di-, and especially tri-isovalerates ("triisovalerin"), isovaleryl salicylic acid or salicylate (salicylic acid isovalerate), ethyl isovalerate, and β -sitosteryl isovalerate. See Figure 1. Hydrolysis of these isovalerate esters *in vivo* releases isovaleric acid and glycerol (glycerin), salicylic acid (an analgesic, anti-inflammatory, and febrifuge), ethanol (ethyl alcohol or common "alcohol," a CNS depressant), and β -sitosterol (a harmless phytosterol), respectively. With the exception of ethyl isovalerate, these esters are non-volatile or

only slightly volatile, thereby minimizing any unpleasant odors. Furthermore, in pure form these esters possess the advantage of having neutral to pleasant odors, in contrast to the extremely unpleasant odors of isovaleric acid and its salts, such as the ammonium, sodium, potassium, and zinc isovalerate salts. Moreover, whereas ethyl isovalerate is a liquid, the glyceryl mono-, di-, and tri-isovalerates, isovaleryl salicylate, and β -sitosteryl isovalerate are expected to be solids at room temperature, thereby facilitating their formulation into various standard solid and liquid oral dosage forms well known in the art, such as tablets (e.g., uncoated tablets, enteric-coated tablets, and film-coated tablets), capsules, gelcaps, powders, concentrates (drops), elixirs, tinctures, and syrups.

In addition to isovaleramide, various substituted amides of isovaleric acid can be prepared by methods well known in the art. See, for example, March, ADVANCED ORGANIC CHEMISTRY: REACTIONS, MECHANISMS, AND STRUCTURE, 4th ed. (John Wiley and Sons 1992). Preferred amides include *N*-ethyl isovaleramide, *N*-methyl isovaleramide, *N,N*-dimethyl isovaleramide, *N*-methyl, *N*-ethyl isovaleramide, *N*-isovaleryl GABA, and *N*-isovaleryl glycine. See, for example, Tanaka et al., *J. Biol. Chem.* 242: 2966 (1967). *N,N*-Diethyl isovaleramide ("Valyl"), although purported to possess CNS depressant (sedative) activity, has recently been shown to possess CNS stimulant (convulsant) properties; see U.S. patent No. 5,506,268 and PCT application WO 94/28,888, *supra*. An amide of isovaleric acid with *p*-aminophenol also can be prepared using standard methods to provide a compound, "isovaleraminophen," which is related structurally to the drug acetaminophen (Tylenol®; see Figure 1). In a manner analogous to that of the isovalerate esters, these substituted amides should be hydrolyzed *in vivo* (in this

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case, via hepatic amidase enzymes), releasing isovaleramide or isovaleric acid.

5 The compounds and preparations discussed above represent alternative forms for delivering isovaleric acid or isovaleramide *in vivo*. In certain cases, such as with isovaleryl salicylic acid and ethyl isovalerate, the pharmacologically active moiety corresponding to the alcohol or phenol portion may be expected to exert its own pharmacological effects. For example, compounds such as "isovaleraminophen" would be expected to exhibit a "Tylenol®-like" effect, as well as the effect expected from the isovaleric acid or isovaleramide moiety. Such novel chemical combinations of a previously known, pharmacologically active alcohol, phenol, or primary or secondary amine with isovaleric acid fall within the scope of the present invention.

10 The pharmaceutical formulations of the present invention can be prepared according to known methods to prepare pharmaceutically useful compositions, whereby active agents are combined in a mixture with a pharmaceutically acceptable carrier. For instance, see REMINGTON'S PHARMACEUTICAL SCIENCES and GOODMAN AND GILMAN'S, both cited above. A composition is said to be in a "pharmaceutically acceptable carrier" if its administration can be tolerated by a recipient patient. Sterile phosphate-buffered saline is one example of a pharmaceutically acceptable carrier. Other suitable carriers (e.g. saline and Ringer's solutions) are well known to those skilled in the art. See, for example, REMINGTON'S PHARMACEUTICAL SCIENCES, *supra*.

25 In general, the dosages of the antispasticity and anticonvulsant agents described herein will vary depending upon such factors as the patient's age, weight, height, sex, general medical condition, and previous medical history. For purposes of therapy, a compound of the present invention and a pharmaceutically acceptable

carrier are administered to a subject in need of such treatment in a therapeutically effective amount. The combination of active agent and carrier is said to be administered in a "therapeutically effective amount" if the amount administered is physiologically significant. An agent is physiologically significant if its presence results in a detectable change in the physiology of a recipient patient. In the present context, for example, an antispasticity agent is physiologically significant if the presence of the agent results in the alleviation of spasticity, while an anticonvulsant agent is physiologically significant if the presence of the agent results in the reduction of the severity, number, or duration of convulsions.

Isovaleramide and related compounds can be administered orally using solid oral dosage forms such as enteric-coated tablets, caplets, gelcaps, or capsules, or via liquid oral dosage forms such as syrups or elixirs. The indicated dosage of isovaleramide and related compounds as antispasticity agents is on the order of 100-1000 mg per dose, and preferably, 300-600 mg per dose. Unit solid oral dosage forms preferably contain about 200-350 mg per tablet or capsule, which typically would be taken 1-2 at a time for a maximum of four times per day, at a dosage of 1-20 mg/kg body weight. Liquid formulations can also be employed with active ingredient compositions so as to provide 1-2 teaspoonfuls per dose. Furthermore, corresponding reduced dosage pediatric chewable and liquid oral dosage forms can also be administered. These compounds also can be added to foods and beverages in the form of drops (with a dropper from a "concentrate" preparation) for oral administration. In addition, compounds such as isovaleramide may be formulated into chewing gum to facilitate oral delivery and absorption.

Alternatively, isovaleramide and related compounds can be administered by injection or other systemic routes, such as transdermal or transmucosal administration, for example, nasally, buccally, or rectally, via suppositories. Oral administration is much more convenient, however, and therefore is preferred.

For use in an oral anticonvulsant composition, the dosage level of active ingredient(s) is on the order of 100-1000 mg per dose, and preferably, 200-600 mg per dose, or 1-20 mg/kg body weight.

In addition to a use in humans, isovaleramide and related compounds can be used, for example, as antispasticity agents or anticonvulsant agents, in animals such as cats, dogs, birds, horses, cattle, mink, poultry, and fish. In such cases the active compound may be administered by injection or other systemic routes, such as transdermal or transmucosal administration (for example, rectal administration via suppositories), or orally by addition to food or drink. As an antispasticity agent, the indicated oral dosage of isovaleramide and/or related compounds per kilogram of body weight of such animals is about 1-1000 mg/kg, depending upon the species of animal and the route of administration. A preferred range for oral dosage is about 200-600 mg/kg body weight.

The indicated oral dosage of isovaleramide and/or related compounds per kilogram body weight as anticonvulsant agents for animals is in the range of about 1-1000 mg/kg, depending upon the species of animal and the route of administration. A preferred range for oral dosage is about 100-600 mg/kg body weight.

The present invention thus contemplates a variety of pharmaceutical compositions containing isovaleramide, isovaleric acid, and/or its pharmaceutically acceptable salts, substituted amides, and alcohol esters as active ingredients that are suitable for oral, parenteral,

transdermal, transmucosal, intranasal, buccal, or rectal administration. Although such compounds may be present as incidental by-products in certain pharmaceutical formulations which are outside the scope of the present invention, the common feature of the present formulations is that isovaleramide, isovaleric acid, and/or its pharmaceutically acceptable salts, substituted amides, and alcohol esters is present in a standardized amount. That is, the pharmaceutical formulations contain a predetermined, chemically defined, and quantifiable amount of at least one of such compounds to enable the determination of the quantity of a particular composition required to achieve the dosage levels described herein.

It is further understood that isovaleramide and/or related compounds can be used in combination with other pharmaceutically active ingredients.

4. DEMONSTRATING THERAPY-IMPLICATING ACTIVITY

The suitability and effectiveness of a given pharmaceutical formulation for the alleviation of a pathology, as discussed above, can be demonstrated using animal models such as (but not limited to) those described below.

(a) The Mutant Spastic Mouse

The mutant spastic mouse is a homozygous mouse that carries an autosomal recessive trait of genetic spasticity. The mouse is normal at birth, and then the mouse develops a coarse tremor, abnormal gait, skeletal muscle rigidity, and abnormal righting reflexes at two to three weeks of age. No structural abnormalities have been found. Rather, the mouse has a deficit of glycine receptors throughout the central nervous system. Drugs that either potentiate the binding or synthesis of GABA, such as valproate and the benzodiazepines, are effective compounds to ameliorate some of the symptoms of spasticity in this model, as well as in humans.

The assessment of spasticity in the mutant spastic mouse can be performed by electrophysiological assessment similar to the EMG recordings described below. One can also, on a more crude scale, measure righting. These mice have an abnormal delayed righting reflex when placed on their backs. Any righting reflex over one second is considered abnormal. Most normal mice cannot even be placed on their backs. Tremor can be evaluated by holding mice by their tails and rating the tremor subjectively by "absent," "slight," "moderate," or "severe." Flexibility is assessed by placing the mouse on a glass object with smoothly rounded grooves and rims. The glass object is lifted about 12 inches above the table and slowly tilted until almost vertical. Normal mice will climb about the object for a minute or more before falling to their feet. Spastic mice usually remain stiffly in one position and soon fall onto their backs. Chai et al., *Proc. Soc. Exptl. Biol. Med.* 109: 491 (1962).

(b) The Acute/Chronic Spinally Transected Rat And The Acute Decerebrate Rat

There are several models of spasticity including the acute decerebrate rat, the acute or chronic spinally transected rat, and the chronically spinal cord-lesioned rat. The acute models, although of proven value in elucidating the mechanisms involved in the development of spasticity, have come under criticism due to the fact that they are acute. The animals usually die or have total recovery from spasticity. The spasticity develops immediately upon intervention, unlike the spasticity that evolves in the human condition of spasticity, which most often initially manifests itself as a flaccid paralysis. Only after weeks and months does spasticity develop in humans. Some of the more chronic-lesioned or spinally transected models of spasticity do post-operatively show flaccid paralysis. At approximately four weeks post-

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lesion/transection, the flaccidity changes to spasticity of variable severity. Although all of these models have their own particular disadvantages and lack of true representation of the human spastic condition, they have provided much information about the nature of spasticity. These models have also provided methods to test various treatment paradigms that have led to similar treatments being tested in humans. Many of these models have also made use of different species, such as cats, dogs, and primates. Baclofen, diazepam, and tizanidine are effective on different parameters of spasticity (EMG recordings, H-reflex, the H/M ratio, mono- and polysynaptic reflexes, clonus, hyperreflexia) in these models.

(c) Primary Observation Irwin Test In The Rat

This method is based on that described by Irwin, *Psychopharmacologia* 13: 222-57 (1968). It is used to detect physiological, behavioral, and toxic effects of a test substance, and indicates a range of dosages that can be used for later experiments. Typically, rats (three per group) are administered the test substance and are then observed in comparison with a control group given vehicle. Behavioral modifications, symptoms of neurotoxicity, pupil diameter, and rectal temperature are recorded according to a standardized observation grid derived from that of Irwin. The grid contains the following items: mortality, sedation, excitation, aggressiveness, Straub tail, writhes, convulsions, tremor, exophthalmos, salivation, lacrimation, piloerection, defecation, fear, traction, reactivity to touch, loss of righting reflexes, sleep, motor incoordination, muscle tone, stereotypies, head-weaving, catalepsy, grasping, ptosis, respiration, corneal reflex, analgesia, abnormal gait, forepaw treading, loss of balance, head twitches, rectal temperature, and pupil diameter. Observations are performed at 15, 30, 60, 120,

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and 180 minutes following administration of the test substance, and also 24 hours later. The test substance is usually administered intraperitoneally (i.p.).

(d) Rotarod Test In The Rat and Mouse

5 This is a test of neurological deficits using the method described by Dunham et al., *J. Am. Pharm. Assoc.* 46: 208-09 (1957). Rats or mice are placed on a rod rotating at a speed of eight turns per minute. The number of animals which drop off the rod before three minutes is counted and the drop-off times are recorded (maximum: 180 sec). Ten rats are studied per group and the test is performed blind. The test compound is administered i.p. 60 min prior to testing. Diazepam, a benzodiazepine, is administered at 8 mg/kg, i.p., as the reference substance. A control group administered the vehicle is also included in the study.

(e) Anticonvulsant Activity

20 There are numerous *in vivo* models involving different kinds of seizures and behavioral effects that are relevant for clinically distinct forms of epilepsy. It therefore is prudent to test for effects in several models, because it may be an oversimplification to suppose that the same mechanism underlies all forms of seizure activity.

25 One useful model is provided by the Frings audiogenic seizure-susceptible mouse, a model of reflex epilepsy. At the time of testing, individual mice are placed into a round Plexiglas chamber and exposed to a sound stimulus of 110 decibels, 11 kHz, for 20 seconds. Animals not displaying tonic hindlimb extensions were considered protected. In addition, the seizure score for each mouse can be recorded as: (1) running for less than 10 seconds; (2) running for greater than 10 seconds; (3) clonic activity of limbs and/or vibrissae; (4) forelimb extension/hindlimb flexion; and (5) hindlimb extension.

5 The average seizure score can be calculated for each group of mice employed in the dose-response study. At each dose, mice are also tested on a rotarod for testing of motor impairment (toxicity). Testing for motor impairment on the rotarod involves placing a mouse for a three-minute trial period on a one-inch diameter rod rotating at six revolutions per minute. If the mouse falls off of the rotating rod three times within the three-minute time period, it is considered a toxic response.

10 (f) Anti-Manic Activity

To assess the possible use of compounds in the treatment of affective mood disorders, one can employ the amphetamine-induced hyperactivity model in rats. In addition to being a test for classical and atypical antipsychotic activity, this procedure has also been proposed as a simple animal model of manic behavior. Costall et al., *Brain Res.* 123: 89-111 (1977).

15 (g) Neurogenic Inflammation Of The Meninges

20 Neurogenic inflammation within the meninges has been proposed as an event in the underlying pathology of migraine headaches. Lee et al., *Brit. J. Pharmacol.* 116: 1661-67 (1995). Compounds are tested for their ability to block the leakage of radiolabeled bovine serum albumin within the dura mater post trigeminal stimulation.

25 (h) Analgesic Properties

30 There are many whole-animal assays for determining analgesic properties, such as writhing, hotplate, tail flick, arthritic pain, paw pressure tests, and the Bennet or Chung models of neuropathic pain. Albe-Fessard et al., in 13 ADVANCES IN PAIN RESEARCH AND THERAPY, pages 11-27 (Raven Press 1990).

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(i) **Therapeutic Benefit Relative To Movement Disorders and Restlessness Syndromes**

Animal models exist for the study of movement disorders and restlessness syndromes, for example, drug-induced akathisia, serotonin syndrome, rotation induced by unilateral nigral lesions. Lloyd & Morselli (1987), *supra*. Additionally, individual case reports of anecdotal efficacy of compounds in humans have been a source for support for these indications. Mellick & Mellick (1995), *supra*; Olson et al., *Am. J. Med.* 102: 60-66 (1997).

The therapeutic effects of isovaleramide, isovaleric acid, and related compounds in various of the assays described above, combined with a general lack of toxicity, make the compounds of the present invention ideal agents for the treatment of the pathologies described above, including spasticity and convulsions/seizures. With this background, the present invention will be understood more readily by reference to the following examples, which are provided for purposes of illustration and are not intended to be limiting of the invention.

Example 1

Use of a Valerian Preparation to Alleviate Symptoms of Spasticity Associated with Multiple Sclerosis

A human female subject, age 42, suffering from one or more symptoms of multiple sclerosis, was experiencing a considerable amount of stress and was experiencing difficulty in getting to sleep and delayed onset of sleep at night. The sleep that did occur was disturbed by stressful dreams and frequent awakening as well. The subject also experienced frequent nighttime painful extensor spasms of the lower extremities that would often awaken the subject from her sleep. The following day,

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these painful extensor spasms resulted in a deep muscle pain (bruising sensation), with muscle/joint stiffness.

5 The subject decided to consume a preparation of valerian that was noted for its sleep-aid properties. The valerian product, "Baldriparan stark N," consists of tablets manufactured in Germany that contain extracts of valerian root, hops, and lemon balm. The coated, pressed tablets each contain 95 milligrams of a dried 70% (v/v) ethanol extract of valerian root, 15 milligrams of a dried 45% (m/m) methanol extract of hops, and 85 milligrams of a dried water extract of lemon balm. Surprisingly, the valerian preparation not only facilitated the onset of sleep and improved the quality of sleep for the subject, but it was also noticed that the painful extensor spasms were alleviated. The subject noted that upon awakening during the night to use the bathroom, she did not experience the painful extensor spasms upon getting out of bed nor the usual stiff-leg sensation. The subject continues to consume the same valerian product for the alleviation of these symptoms on an as-needed basis (*prn* or *pro re nata*) and continues to experience relief.

Example 2

25 *Use of a Valerian Preparation to Alleviate Symptoms of Spasticity Associated with Spinal Cord Injury*

30 A human male subject, age 38, suffers symptoms of spasticity (hyperreflexia, tendon jerks, and extensor spasms) that evolved from an earlier injury to the spinal cord. All of these symptoms interrupt and decrease the quality of sleep experienced by this individual. Upon taking the same German-made preparation of valerian described in Example 1, the subject noted considerable improvement in the quality of sleep as well as a significant reduction in night-time extensor spasms. 35 This subject continues to consume the preparation on an

as-needed (prn) basis for the alleviation of the symptoms described above.

Example 3

Isovaleramide Antispasticity Tests

(1) Assessment of Spasticity in Chronic Spinally Transected Rats

In these studies, male albino Holtzman-derived rats (Harlan Sprague-Dawley Laboratories) weighing 270-530 grams were used as subjects. The animals were housed independently and had continuous access to food and water throughout the experiments. All procedures were reviewed and approved by the Institutional Animal Care and Use Committee. Animals were anesthetized using a mixture of isoflurane and oxygen at a flow rate of 4 liters/minute. The rats were then placed in a stereotaxic frame and anesthesia was maintained. An incision was made so that the paraspinal muscles could be retracted and a laminectomy performed between T6-T9. A one- to two-millimeter portion of the spinal cord was removed by evacuation and replaced with Gel foam to reduce bleeding, after which the incision was closed in layers.

Following the transection, rats were placed in a room in which the ambient temperature was raised to about 80°F with a space heater to maintain body temperature. On the following morning post-surgery, the hindquarters of the spinalized rats were bathed and their urine expressed manually by applying pressure to their bladders. Experiments were conducted between 21 and 28 days after surgery. For the first two weeks, these rats were given 0.25 ml of the antibiotic Sulfatrim Pediatric Suspension orally to prevent bladder infection. A commercial antibiotic cream was applied to any part of the skin that showed signs of decubitus lesions. Within approximately two weeks, all animals regained bladder control and were no longer given antibiotic treatment. Advokat, Brain

Res. 684: 8 (1995). Assessment of spasticity was performed before and after drug treatment such that each animal served as its own control.

5 Initial assessment of spasticity was performed by the subjective scoring method of rating the resulting spasticity response elicited with an innocuous stimulus, i.e., a metal probe, that was pressed against the lower abdomen at four specific sites. The spastic reaction was evaluated for each of the four trials using a scale ranging from zero (no spastic response in all four trials) to four (a maximum, tonic-clonic reaction elicited in all four trials). All spasticity scores, pre- and post-treatment, were transformed to indicate the percent spasticity such that a score of 0/4 = 0%, 1/4 = 10
15 25%, etc. These raw or normalized scores were analyzed with a one-way repeated measures ANOVA.

As shown in Figure 2, isovaleramide at a dose of 300 mg/kg, i.p., was efficacious at 15, 30, 60, and 120 minutes post-administration in reducing the spasticity scores (45-65%). By the next day, i.e., by 1440 minutes (24 hours), the spasticity scores had essentially returned to baseline values. No overt behavioral toxicity or motor impairment was observed at this dose. The rats were alert and able to grasp with their non-paralyzed front paws as were the control, untreated rats.

25 With reference to Figure 3, the polysynaptic flexor-reflex responses, to test stimuli which activate high-threshold afferents, were recorded as EMG activity from the ipsilateral hamstring muscle. Supramaximal electric shocks were applied to the hindpaw, and recording electrodes were placed in the biceps femoris semitendinosus muscle. Five sets of stimuli were made at each time point. The flexor reflex was recorded, in both the pre-drug and the post-drug periods, every 30 minutes once a stable baseline response was achieved. See Hao et al., Eur. J. Pharmacol. 191: 407 (1990).

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Thus, the responses were determined in spinalized rats by observing the flexor-reflex response (Figure 3) before treatment and at each of 30, 60, 90, and 120 minutes following administration of isovaleramide (300, 600, and 1200 mg/kg p.o.), baclofen (10 mg/kg s.c.) and vehicle (water, 12 ml/kg p.o.), respectively.

Isovaleramide at all doses was shown to reduce the magnitude of the flexor-reflex responses, at all time points in a chronic spinalized rat, with spasticity. In this model, no change in the H/M reflex was observed with either baclofen or isovaleramide.

In Figure 4, the responses from Figure 3 are converted to a total-area-under-the-curve format, covering the entire, two-hour measurement period. All drug-treated groups differed significantly from vehicle ($p < 0.05$), based on a one-way analysis of variance. Between the drug-treated groups, no differences were found in total reduction of the flexor reflex over the two-hour period (pairwise multiple comparison, Student-Newman-Keuls method).

(2) Primary Observation Irwin Test in the Rat

Administered i.p. in the rat, isovaleramide induced no changes from saline-injected controls at doses up to 256 mg/kg. At 512 mg/kg, slight sedation from 60 to 120 minutes, loss of traction (observed in only one of three rats) at 120 minutes, and decreased muscle tone from 60 to 120 minutes were observed. At 1024 mg/kg, marked sedation up to 30 minutes was observed, becoming moderate up to 120 minutes, then slight at 180 minutes. Decreased fear was also observed at this dose up to 30 minutes and in one of three rats up to 120 minutes. Decreased reactivity to touch up to 120 minutes, decreased muscle tone up to 180 minutes, slight hypothermia up to 120 minutes, and an abnormal gait (rolling) from 60 to 80 minutes were also observed at this dose. Loss of

-32-

grasping and loss of righting reflex occurred, in one of three rats, at 15 minutes at this dose.

(3) Rotarod Test in the Rat and the Frings Mouse

Isovaleramide, administered at doses of 128, 256, and 512 mg/kg (i.p.) 60 minutes before a test on the rotarod, did not significantly affect rotarod performance in the rat. See Table 1. In contrast, diazepam dose-dependently decreased rotarod performance.

Table 1

Effects of Isovaleramide and Diazepam in the Rotarod Test in the Rat

Dose of: Isovaleramide (mg/kg) ^a	Number ^b of Rats Falling	Drop-Off Time (sec)		
		Mean ± S.E.M.	t value	% change from control
0	5	135.5 ± 18.0	-	-
128	6	134.5 ± 20.7 ^c	0.036	-1%
256	7	98.4 ± 23.3 ^c	1.261	-27%
512	7	115.9 ± 20.5 ^c	0.717	-14%
Diazepam (mg/kg) ^a				
4	9	55.8 ± 20.6 ^d	2.909	-59%
8	10+ ^e	16.3 ± 6.4 ^f	6.222	-88%

^a Isovaleramide and diazepam were administered i.p. 60 minutes before the rotarod test.

^b Ten rats per group.

^c Not significant according to Student's t Test.

^d p<0.01 according to Student's t Test.

^e p<0.05 according to Fisher's Exact Test.

^f p<0.001 according to Student's t Test.

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Isovaleramide, administered at doses up to 150 mg/kg (i.p.) 15 minutes before a test on the rotarod in the Frings mouse, did not affect performance significantly. In contrast, doses of 300 mg/kg, 600 mg/kg, and 1000 mg/kg (i.p.) decreased rotarod performance in 1/8, 4/8, and 8/8 of Frings mice tested, respectively.

Example 4

Anticonvulsant Activity in the Frings Audiogenic Seizure-Susceptible Mouse Model of Epilepsy

The results of Table 2 demonstrate isovaleramide anticonvulsant activity in this animal model of epilepsy. Isovaleramide also displayed a quick onset and a relatively short duration of action. Anticonvulsant activity was noted as early as 15 minutes, but decreased substantially by two hours. All quantitative studies therefore were conducted at 15 minutes. At this time point, the median effective dose (ED_{50}) for protection against tonic extension was 126 mg/kg, i.p. In addition, a dose-dependent reduction in seizure score was observed at this time point. At doses markedly higher than those providing anticonvulsant activity (>300 mg/kg), animals treated with isovaleramide displayed behavioral toxicity that was characterized by their inability to maintain their balance on the rotarod. No notable toxicity was observed at doses less than 300 mg/kg. The median toxic dose (TD_{50}) for rotarod impairment was 531 mg/kg, i.p. Thus, the calculated protective index (TD_{50}/ED_{50}) was about 4.2.

Therefore, despite the relatively low potency of isovaleramide in this model, it still displayed a relatively good separation between activity and toxicity. Isovaleramide thus had a surprising and unexpected efficacy, based on existing structure-activity relationships for amides and their corresponding acids, as an anticonvulsant in the Frings audiogenic seizure-

susceptible mouse model of reflex epilepsy. The activity profile of isovaleramide is similar to that of the broad-spectrum anticonvulsant, sodium valproate. Compounds similar in structure to valproate as well as isovaleric acid have been shown in previous literature to elevate GABA levels throughout the CNS. It is this function, primarily, that the anticonvulsant activity of valproate is attributed to, although other mechanisms have been suggested. Isovaleric acid, on the other hand, was found to be inactive as an anticonvulsant, although it was reported to elicit a slight increase in GABA levels in the brains of mice. For example, see Löscher et al., *Neuropharmacology* 24: 427 (1985); Keane et al., *loc. cit.* 22: 875 (1983); Keane et al., *Pharmacol. Res. Commun.* 17: 547 (1985).

-35-

Table 2

Effect of Isovaleramide on the Audiogenic Seizure
Susceptibility of Frings Mice Following Intraperitoneal
Administration

Dose of Isovaleramide (mg/kg, i.p.)	Seizure Score \pm S.E.M.	Number ^a Protected of Eight Mice Tested	Number ^a Showing Toxicity of Eight Mice Tested
75	4.4 \pm 0.6	1	0
112.5	4.0 \pm 0.6	2	0
150	2.0 \pm 0.6	6	0
300	1.0 \pm 0	8	1
600	-		4
1000	-		8
ED ₅₀ for protection: 126 mg/kg (98.8 - 168 ^b)			
TD ₅₀ : 531 mg/kg (372 - 711 ^b)			

^a Measured at 15 minutes.

^b 95% confidence interval.

In general, the historical literature on the structure-activity relationships of anticonvulsant activity around compounds similar to valproate have taught away from simple, unsubstituted compounds such as isovaleramide. It is thus a surprising and unexpected observation that isovaleramide has demonstrated an efficacy profile similar to that of valproate in the Frings audiogenic seizure-susceptible mouse model and a similar separation of activity between efficacy and toxicity as measured by rotarod performance. These observations indicate that isovaleramide is an effective therapeutic agent as a broad-spectrum anticonvulsant. Isovaleramide is known for its relative lack of toxicity in mutagenicity and cytotoxicity tests. See U.S. patent No. 5,506,268 and PCT application WO 94/28,888. On the

other hand, valproate has long been noted for its hepatotoxicity-causing profile. For example, see Löscher et al., *Neuropharmacology* 24: 427 (1985).

5 Although the foregoing refers to particular preferred
embodiments, it will be understood that the present
invention is not so limited. It will occur to those of
ordinary skill in the art that various modifications may
10 be made to the disclosed embodiments and that such
modifications are intended to be within the scope of the
present invention, which is defined by the claims below.

15 All publications and patent applications mentioned
in this specification are indicative of the level of
skill of those in the art to which the invention
pertains. All publications and patent applications are
herein incorporated by reference to the same extent as if
each individual publication or patent application were
specifically and individually indicated to be
incorporated by reference in its entirety.

What Is Claimed Is:

1. Use of a compound selected from the group consisting of isovaleric acid, a pharmaceutically acceptable salt of isovaleric acid, a pharmaceutically acceptable ester of isovaleric acid, and a pharmaceutically acceptable amide of isovaleric acid in the preparation of a pharmaceutical formulation for use in a method of treating a pathology that is ameliorated by a mild depression of CNS activity, whereby at least one symptom of said pathology is alleviated.

2. A use according to claim 1, wherein said pathology is selected from the group consisting of an affective mood disorder, convulsions, a central neuropathic pain syndrome, a headache, and a restlessness syndrome.

3. A use according to claim 2, wherein said pathology is convulsions.

4. A use according to claim 1, wherein said pathology is ameliorated by a centrally mediated decrease in muscle tone.

5. A use according to claim 4, wherein pathology is spasticity.

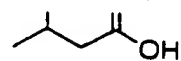
6. A use according to claim 1, wherein said composition is isovaleramide.

7. Use of an extract of Valerianaceae, cramp bark, black haw, or hops in the preparation of a pharmaceutical formulation for use in a method of treating a symptom of spasticity, wherein said extract comprises at least one compound that is hydrolyzed *in vivo* to yield isovaleric acid or isovaleramide.

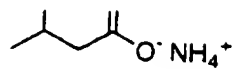
8. A treatment method comprising the step of administering, to a patient suffering from a pathology that is ameliorated by a mild depression of CNS activity, a therapeutically effective amount of a pharmaceutical formulation comprising a pharmaceutically acceptable carrier and a composition selected from the group consisting of isovaleric acid, a pharmaceutically acceptable salt of isovaleric acid, a pharmaceutically acceptable ester of isovaleric acid, and a pharmaceutically acceptable amide of isovaleric acid, whereby at least one symptom of said pathology is alleviated.

9. A method for alleviating a symptom of spasticity in a subject in need of such treatment, comprising the step of administering a therapeutically effective amount of an extract of Valerianaceae, cramp bark, black haw or hops, wherein said extract comprises at least one compound that is hydrolyzed *in vivo* to yield isovaleric acid or isovaleramide.

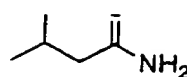
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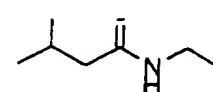
Isovaleric acid



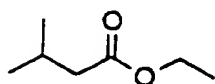
Ammonium isovalerate



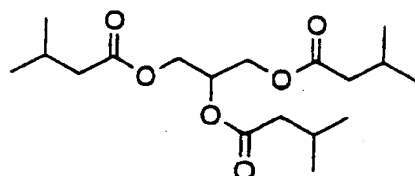
Isovaleramide



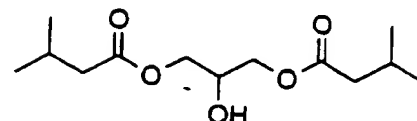
N-Ethyl isovaleramide



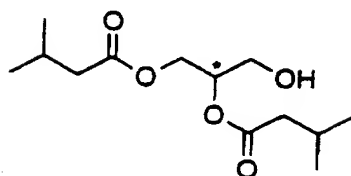
Ethyl isovalerate



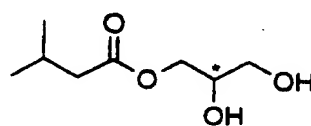
Glycerol triisovalerate ("Triisovalerin")



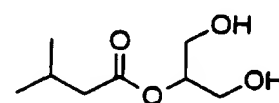
Glycerol-1,3-diisovalerate



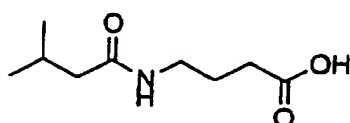
Glycerol-1,2-diisovalerate



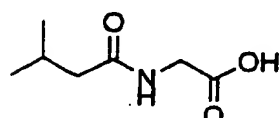
Glycerol-1-monoisovalerate



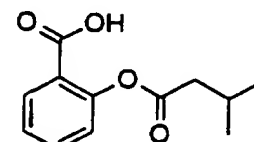
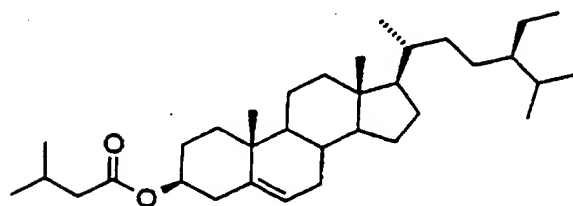
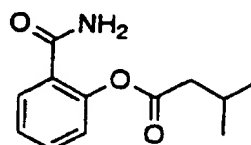
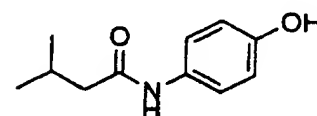
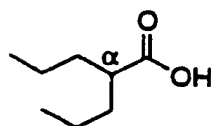
Glycerol-2-monoisovalerate



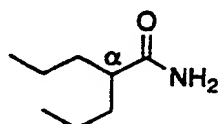
N-Isovaleryl GABA



N-Isovaleryl glycine

Isovaleryl salicylic acid
("I.S.A."); salicylic acid
isovalerate β -Sitosteryl isovalerateIsovaleryl salicylamide
(salicylamide isovalerate
ester)"Isovaleraminophen"
[N-(4-hydroxyphenyl)-
isovaleramide]

Valproic acid



Valpromide

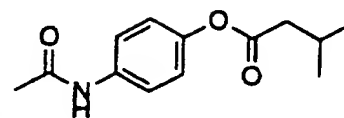
Isovaleryl-acetaminophen
ester

FIGURE 1.

-2/4-

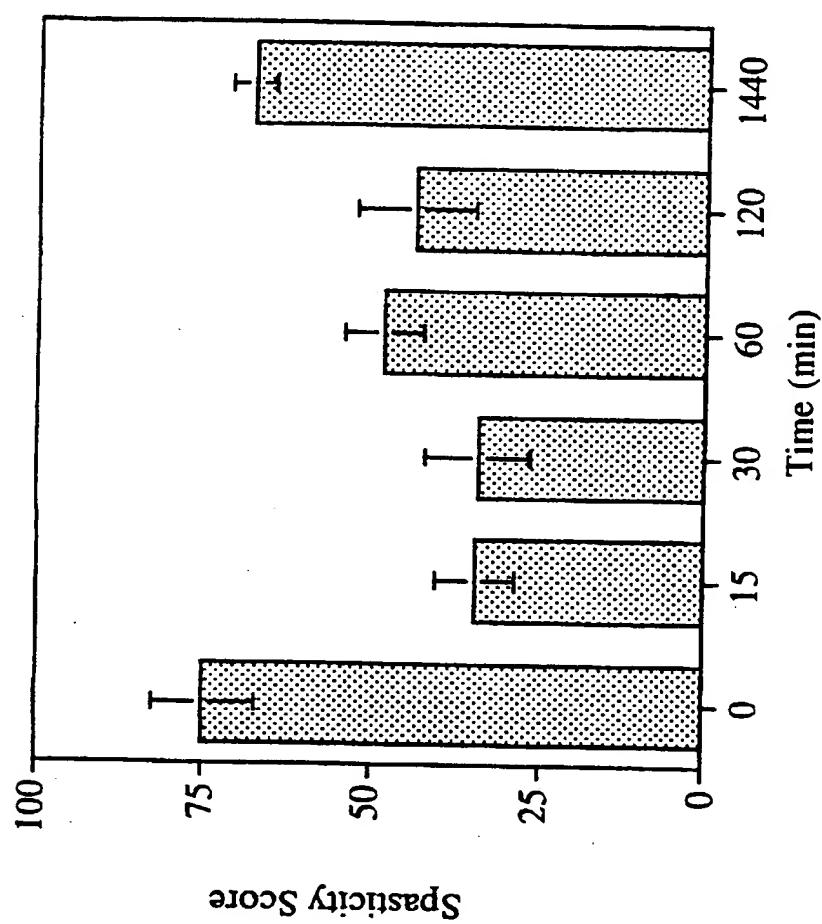


Figure 2. Isovalemide (300 mg/kg, i.p.) reduces the spasticity scores in chronic spinalized rats.

-3/4-

The Flexor Reflex in the Chronic Spinalized Rat

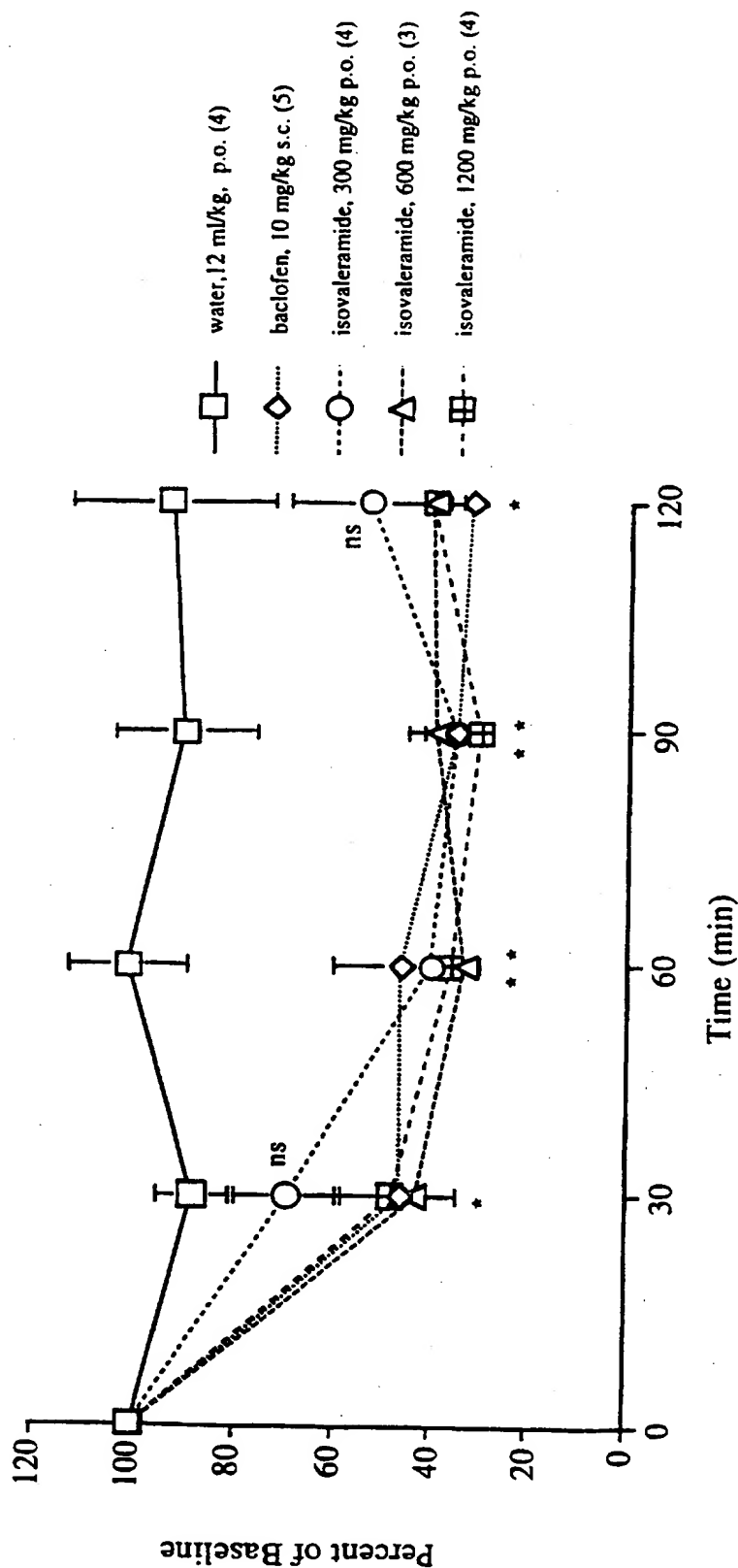


Figure 3. Isovaleramide produces a dose- and time-dependent reduction in the magnitude of the flexor reflex in chronic spinalized rats. * $p < 0.05$, ** $p < 0.01$, NS = not significant (1-way ANOVA and post-hoc Dunnett's t-test).

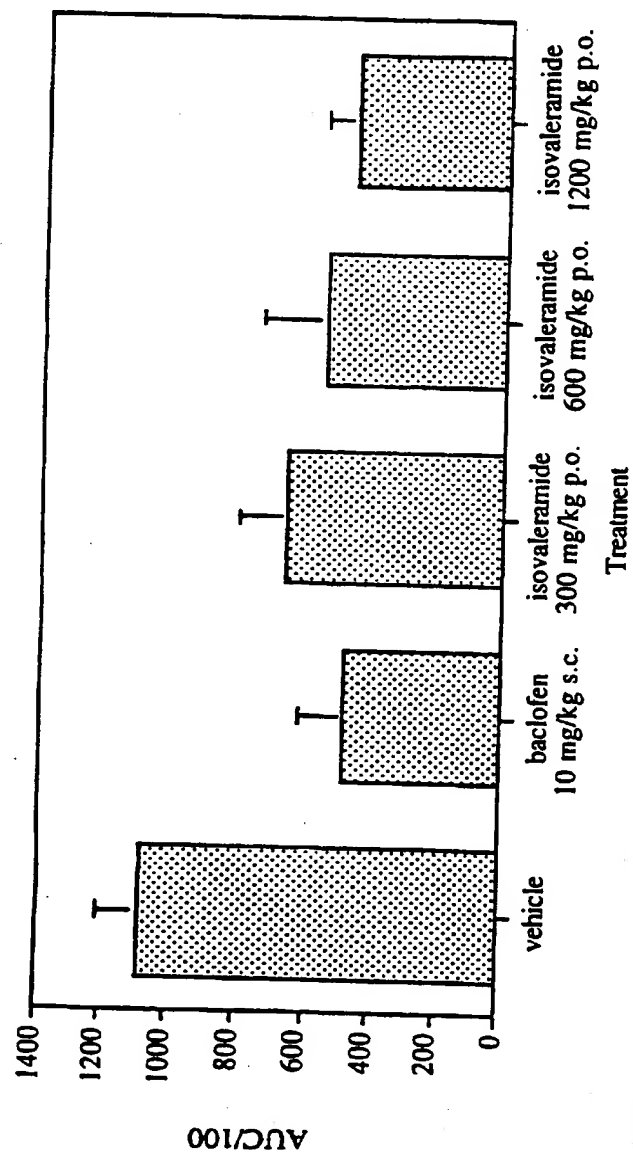


Figure 4. Isovaleramide and baclofen produce a similar reduction of the flexor reflex in chronic spinalized rats. The responses from Figure 3 are converted to a total area-under-the-curve for the 2 hour measurement. All drug-treated groups are significantly different from the vehicle-treated group ($p < 0.05$, One Way Analysis of Variance).

INTERNATIONAL SEARCH REPORT

Internat. Application No

PCT/US 97/15272

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 A61K31/16 A61K35/78

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KEANE ET AL.: "EFFECT OF VALPROATE ON BRAIN GABA : COMPARISON WITH VARIOUS MEDIUM CHAIN FATTY ACIDS" PHARMACOL. RES. COMMUN., vol. 17, no. 6, 1985, pages 547-555, XP002049841 in particular table 1 page 551 see the whole document	1-3
X	HAZELHOFF ET AL.: "Antispasmodic effects of Valeriana Compounds: An in-Vivo and in-Vitro Study on the Guinea-Pig Ileum" ARCH. INT. PHARMACODYN., vol. 257, no. 2, 1982, pages 274-287, XP002049842 see the whole document	1,4,7

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"Z" document member of the same patent family

Date of the actual completion of the international search

16 December 1997

Date of mailing of the international search report

21. 01. 98

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A. Jakobs

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 97/15272

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MORAZZONI ET AL.: "Valeriana officinalis: traditional use and recent evaluation of activity" FITOTERAPIA, vol. 66, no. 2, 1995, pages 99-112, XP002049843 see the whole document	1,2,5, 7-9
X	BALANSARD ET AL.: "Critères de choix d'un extrait de Viburnum, Viburnum Prunifolium L., en fonction de ses actions toniveineuse et spasmolytique." PLANT MED. PHYTOTHER., vol. 17, no. 3, 1983, pages 123-132, XP002049844 see the whole document	7-9
X	FURNADJIEV G.: "PHYTOTHERAPEUTIC AND EXPERIMENTAL DATA ON SOME MEDICINAL PLANTS WITH SPASMOlyTIC EFFECT AND THEIR APPLICATION IN GASTROINTESTINAL DISEASES" PROBL. VATR. MED., vol. 8, no. 3, 1980, pages 79-86, XP002049845 see abstract	7-9
X	WAGNER ET AL.: "Ueber die spasmolytische Wirkung des Badrians" PLANTA MED., vol. 37, no. 1, 1979, pages 84-86, XP002049846 see the whole document	7-9
X	US 3 485 851 A (THIES PETER WILLIBRORD) 23 December 1969 see column 1, li 36-39 see the whole document	1-3,5, 7-9
X	DE 22 30 626 A (SCHAETTE ROLAND DR) 10 January 1974 see in particular page 1 see the whole document	7-9
X	WO 94 28888 A (NPS PHARMA INC ;BALANDRIN MANUEL F (US); WAGENEN BRADFORD C VAN (U) 22 December 1994 in particular pages 12-16 see the whole document	1,2,6-9

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INTERNATIONAL SEARCH REPORT

Internal Application No
PCT/US 97/15272

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>TAILLANDIER ET AL.: "Recherches dans la série dipropylacétique XII. Acides et alcools aliphatiques ramifiés anticonvulsivants"</p> <p>EUR. J. MED. CHEM., vol. 10, no. 5, 1975, POC038, pages 453-462, XP002049847 see in particular table 1, page 455 see the whole document</p> <p>---</p>	1-3
X	<p>WEISS R.F.: "Herbal Medicine"</p> <p>1988 , AB ARCANUM , GOTHENBURG, SWEDEN XP002049848 00014 Chapter 11, pages 280-286, 290 and chapter 12 page 307, column 1.</p> <p>---</p>	7-9
X	<p>BÉZANGER-BEAUQUESNE ET AL.: "Plantes médicinales des régions tempérées"</p> <p>1980 , MALOINE S.A. , PARIS XP002049849 00002 see pages 78, 363, 364 and 365</p> <p>-----</p>	7-9

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
see FURTHER INFORMATION sheet PCT/ISA/210
2. ☒ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This international search report has not been established in respect of
-----the following reasons:

Claims Nos.: 1-4

because they relate to subject matter not required to be searched by this Authority, namely:

In view of the large number of compounds, which are defined by the general definition(s)/formulae used in claims 1-4, the search had to be restricted for economic reasons. The search was limited to the compounds for which pharmacological data was given and / or the compounds mentioned in the claims, and to the general idea underlying the application. (see Guidelines, chapter III, paragraph 2.3)

Claims Nos.: -

because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

In view of the large number of compounds, which are defined by the general definition(s)/formulae used in claims 1-4, the search had to be restricted for economic reasons. The search was limited to the compounds for which pharmacological data was given and / or the compounds mentioned in the claims, and to the general idea underlying the application. (see Guidelines, chapter III, paragraph 2.3)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 97/15272

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 3485851 A	23-12-69	DE 1617555 A DK 115651 B NL 6705435 A	01-04-71 27-10-69 20-10-67
DE 2230626 A	10-01-74	NONE	
WO 9428888 A	22-12-94	US 5506268 A AU 7100694 A CN 1127472 A EP 0706385 A JP 9501908 T	09-04-96 03-01-95 24-07-96 17-04-96 25-02-97



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : A61K 31/16, 35/78	A1	(11) International Publication Number: WO 98/08498 (43) International Publication Date: 5 March 1998 (05.03.98)
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(21) International Application Number: **PCT/US97/15272**(22) International Filing Date: **29 August 1997 (29.08.97)**(30) Priority Data:
60/025,050 **30 August 1996 (30.08.96)** **US**

(60) Parent Application or Grant

(63) Related by Continuation

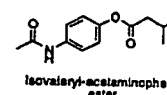
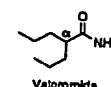
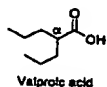
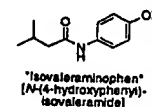
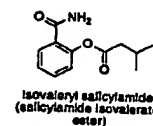
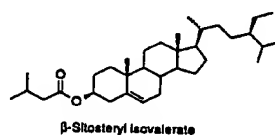
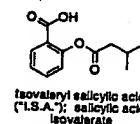
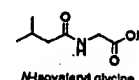
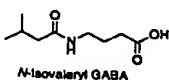
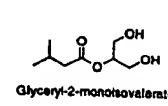
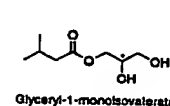
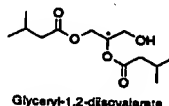
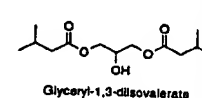
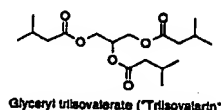
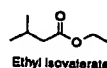
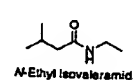
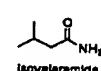
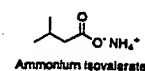
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(57) Abstract

Preparations and extracts of valerian, as well as isovaleramide, isovaleric acid, and its pharmaceutically acceptable salts, esters, and substituted amides, exhibit clinically significant pharmacological properties which implicate a treatment for a variety of pathological conditions, including spasticity and convulsions, which are ameliorated by effecting a mild depression of CNS activity. The compositions in question generally are non-cytotoxic and do not elicit weakness or sedative activity at doses that are effective for the symptomatic treatment of such pathological conditions.



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TREATMENT OF SPASTICITY, CONVULSIONS BY ISOVALERIC ACID DERIVATIVES CNS
DEPRESSANTS**BACKGROUND OF THE INVENTION**

5 The present invention relates to treating pathological conditions, such as spasticity and convulsions, the symptoms of which are alleviated by a mild depression of activity in the central nervous system (CNS), without producing undesirable excessive sedation or muscle weakness in animal subjects, including humans. More particularly, the invention relates to the therapeutic use of isovaleramide, isovaleric acid, and related compounds in patients suffering from pathologies of this nature.

10 Many agents currently employed in the treatment of pathologies such as spasticity and convulsions display troubling side-effect profiles which limit their long-term clinical utility. Among these agents, for example, 15 are the benzodiazepines, which can cause cognitive blunting. Two other agents are valproate, which exhibits hepatotoxicity, and baclofen, which can produce excessive muscle weakness and sedation, limiting the therapeutic potential for both drugs.

SUMMARY OF THE INVENTION

20 Accordingly, it is an object of the present invention to provide a therapeutic approach for the treatment of various pathologies by effecting a mild depression in CNS activity without producing excessive sedation, muscle 25 weakness, fatigue, or hepatotoxicity.

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5 It also is an object of the present invention to provide a method for alleviating one or more symptoms associated with a condition, such as spasticity, that is ameliorated by means of a centrally mediated decrease in muscle tone.

It is another object of the present invention to provide a novel anticonvulsant therapy.

10 In accomplishing these and other objectives, there has been provided, according to one aspect of the present invention, a use of a compound selected from the group consisting of isovaleric acid, a pharmaceutically acceptable salt of isovaleric acid, a pharmaceutically acceptable ester of isovaleric acid, and a pharmaceutically acceptable amide of isovaleric acid in
15 the preparation of a pharmaceutical formulation for use in a method of treating a pathology that is ameliorated by a mild depression of CNS activity, whereby at least one symptom of said pathology is alleviated. Thus, the present invention also contemplates a treatment method comprising the step of administering, to a patient
20 suffering from a pathology that is ameliorated by a mild depression of CNS activity, a therapeutically effective amount of a pharmaceutical formulation comprising a pharmaceutically acceptable carrier and a composition selected from the aforementioned group of agents.

25 Pursuant to one embodiment of the invention, the treated pathology is an affective mood disorder, convulsions, a central neuropathic pain syndrome, a headache, or a restlessness syndrome. For another
30 embodiment, the pathology in question is ameliorated by a centrally mediated decrease in muscle tone, and is illustrated by spasticity.

35 In accordance with another aspect of the present invention, a use is provided for an extract of Valerianaceae, cramp bark, black haw, or hops in the preparation of a pharmaceutical formulation for use in a

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method of treating a symptom of spasticity, where the extract comprises at least one compound that is hydrolyzed *in vivo* to yield isovaleric acid or isovaleramide. By the same token, the present invention provides a method for alleviating a symptom of spasticity in a subject in need of such treatment, comprising the step of administering a therapeutically effective amount of an extract as described above.

Other objects, features, and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating preferred embodiments of the present invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts the structures of various compounds, including isovaleramide.

Figure 2 portrays the effect of isovaleramide (at 300 mg/kg, *i.p.*) on gross observational spasticity scores elicited by a metal probe applied to the abdomen in the chronic spinalized rat. Each rat served as its own control; there were three rats per group. The bar at time zero represents pre-treatment control values.

Figure 3 illustrates a dose- and time-dependent reduction of the flexor reflex, an electrophysiological measure of spasticity, in the chronic spinalized rat. The effects of isovaleramide (300, 600, and 1200 mg/kg *p.o.*), baclofen (10 mg/kg *s.c.*), and vehicle (water, 12 ml/kg *p.o.*) are shown at pre-treatment (time zero) and at 30, 60, 90, and 120 minutes post-administration. At all

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5 doses, isovaleramide caused a significant decrease in the magnitude of the flexor reflex, comparable to that observed with baclofen. Statistical significance was assessed by one-way analysis of variance (ANOVA) and post-hoc Dunnett's t-test: $p < 0.05$ (*); $p < 0.01$ (**); NS = not significant.

10 Figure 4 shows that isovaleramide and baclofen, a known antispasticity agent, produced a similar reduction of the flexor reflex in the chronic spinalized rat. The responses from Figure 3 were converted to a total-area-under-the-curve for the two-hour measurement. All drug-related groups were significantly different from the vehicle ($p < 0.05$, ANOVA).

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

15 1. OVERVIEW

20 The inventors have discovered that isovaleric acid and its pharmaceutically acceptable salts, amides such as isovaleramide, and alcohol esters such as ethyl isovalerate and β -sitosteryl isovalerate can be administered *in vivo* to effect a mild depression of CNS activity. That is, these agents depress CNS activity, by enhancing inhibitory (or decreasing excitatory) neurotransmission centrally, without complete suppression of all activity. Pursuant to the present invention, therefore, a subject who receives such an agent is not overtly sedated, anesthetized, or paralyzed in the context, for example, of decreasing seizures (no anesthesia), decreasing muscle tone (no paralysis), eliciting a calmative effect (no sedation), or ameliorating an ambulatory syndrome such as spasticity (no weakness or flaccidity).

30 A number of pathologies, exemplified by affective mood disorders, headaches (chronic, cluster, migraine),

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restlessness syndromes, neuropathic pain, movement disorders, spasticity, and convulsions, have at least one symptom that is alleviated by a mild CNS depression. Accordingly, an individual who suffers from such a pathology is a candidate for therapy that entails, pursuant to the present invention, the individual's receiving a pharmaceutical formulation of isovaleramide, isovaleric acid, or a related compound.

It is believed that the compounds of the present invention act via a GABAergic mechanism and, hence, bear a pharmacological similarity to known drugs which are considered to enhance central GABAergic neurotransmission. Like many of the extant drugs, such as the barbiturates, the benzodiazepines, gabapentin, valproate, vigabatrin, and progabide, the compounds of the present invention are effective in treating pathological conditions, illustrated by those mentioned above, that are thought to arise from a defect in the regulation of inhibitory (GABA- and/or glycine-related) neurotransmission. This regulation may occur by a direct or modulatory effect at CNS receptors or by impact on a metabolic pathway which heightens GABA or glycine levels and/or which reduces levels of an excitatory neurotransmitter like glutamate. See Ruggero *et al.*, in *ANTIEPILEPTIC DRUGS* (4th ed.), pages 581-88 (Raven Press 1995); Nogrady, *MEDICINAL CHEMISTRY: A BIOCHEMICAL APPROACH* (2d ed.), pages 225-39 (Oxford University Press 1988); Fonnum and Morselli, respectively, in *PSYCHOPHARMACOLOGY: THE THIRD GENERATION OF PROGRESS*, pages 173-82 and 183-95 (Raven Press 1987). Despite a pharmacological analogy to the known drugs mentioned above, however, the present invention surprisingly does not engender the disadvantageous side effects associated with conventional drug therapies in this area, such as the hepatotoxicity that arises with valproate administration.

2. **EXEMPLARY PATHOLOGIES AMELIORATED
BY A MILD DEPRESSION OF CNS ACTIVITY**

5 **SPASTICITY:** "Spasticity is frequently defined as an upper [i.e., CNS] motor neuron disorder characterized by a velocity dependent increase in tonic stretch reflexes (muscle tone) with exaggerated tendon jerks resulting from hyperexcitability of the stretch reflex." Lance, Symposia synopsis in SPASTICITY - DISORDERED MOTOR CONTROL, Feldman et al. (eds.) (1980). An increase in
10 tonic stretch reflexes, however, is only one of the many symptoms present in a disordered motor function caused by an upper neuron lesion in a variety of neurological disorders; thus, such a disordered motor function is variable in its etiology and presentation.

15 Major disease states and conditions associated with spasticity include multiple sclerosis, cerebral palsy, stroke, trauma or injury to the spinal cord, and closed head trauma. There are "positive symptoms" that can occur with spasticity, such as the Babinski response,
20 painful flexor or extensor spasms, increased or exaggerated deep tendon reflexes, and clonus. Other symptoms, referred to as "negative symptoms," include weakness, fatigue, lack of dexterity, and paralysis. It is the combination of these positive and negative signs
25 and symptoms that is denoted clinically as "spastic paresis" (spastic paralysis). Pain, impairment of sleep, and various degrees of loss of general motor function are also associated with spasticity.

30 The pathological states observed in spasticity are fundamentally different at the physiological level from the commonly experienced acute muscular aches, strains, and sprains that occur from a localized external insult to a particular muscle, i.e., outside of or peripheral to
35 the CNS. These pathological states also are different from the relatively common involuntary spasms of smooth muscle, such as vascular spasms, bladder spasms, and

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bronchial spasms. Such non-spastic (non-CNS), peripheral or localized symptoms are commonly treated with so-called "antispasmodic" or "spasmolytic" agents, but these generally are not useful in treating spasticity. Cedarbaum & Schleifer, "Drugs for Parkinson's Disease, Spasticity and Acute Muscle Spasms," in GOODMAN AND GILMAN'S THE PHARMACOLOGICAL BASIS OF THERAPEUTICS, 8th ed. [hereafter GOODMAN AND GILMAN'S], pages 463-484 (Pergamon Press 1990).

The pharmaceutical formulations employed in accordance with the present invention can effect a centrally mediated decrease in muscle tone and, hence, are useful for the acute or chronic alleviation of one or more symptoms of spasticity. In this context, "spasticity" refers to a heightened tone of skeletal muscle which is manifested by symptoms such as (but not limited to) painful flexor or extensor spasms, increased or exaggerated deep tendon reflexes, hyperreflexia, loss of dexterity, muscular weakness, exaggerated tendon jerks, and clonus. The phrase "antispasticity agent" refers here to a composition that is useful for the symptomatic treatment of spasticity, as demonstrated by the alleviation of at least one of the following manifestations of spasticity: painful flexor or extensor spasms, increased or exaggerated deep tendon reflexes, hyperreflexia, loss of dexterity, muscular weakness, exaggerated tendon jerks, and clonus. Accordingly, the "alleviation" of spasticity refers here to the lessening of one or more symptoms of spasticity, including but not limited to painful flexor or extensor spasms, increased or exaggerated deep tendon reflexes, hyperreflexia, loss of dexterity, muscle weakness, exaggerated tendon jerks, and clonus.

Spasticity is associated with multiple sclerosis, stroke, head trauma, spinal cord injuries, cerebral palsy, and other neurodegenerative diseases, disorders,

and conditions. Spasticity is distinct from acute muscle spasms, which may be associated with a variety of conditions different from those leading to spasticity. These acute muscle spasm-causing conditions include trauma, inflammation, anxiety, and/or pain.

The difference between spasticity and acute muscle spasms is illustrated by the fact that agents useful for the treatment of muscle spasms are not useful for treating spasticity associated with chronic neurological diseases. Cedarbaum & Schleifer (1990), *supra*. Likewise, agents used heretofore to treat spasticity associated with chronic neurological disorders have not been employed in treating acute muscle spasms, except for the benzodiazepines, such as diazepam (Valium®), which are recognized also to have muscle-relaxant activity as well as anxiolytic and analgesic properties. By contrast, the present invention achieves a centrally mediated decrease in muscle tone which, in turn, addresses the particular symptoms of spasticity.

CONVULSIVE DISORDERS: Due to the widespread availability of reasonably predictive and experimentally accessible animal models of convulsant states, a number of clinically useful anticonvulsants have been prepared and developed. For example, see Cereghino *et al.*, "Introduction," in *ANTIEPILEPTIC DRUGS*, 4th ed., pages 1-11 (Raven Press 1995). "In many patients, seizures can be controlled with currently available antiepileptic drugs, but 25 to 30 percent of patients continue to have seizures despite optimal therapy, while many others experience unacceptable side effects." Dichter *et al.*, *Drug Therapy* 334: 1583 (1996).

Thus, many anticonvulsants in clinical use are plagued by the occurrence of significant side effects, including troublesome daytime sedation, muscular weakness, tolerance, gingival hyperplasia, blood dyscrasias, and potentially fatal hepatotoxicity. Many

of these side effects are especially of concern in the clinical management (treatment) of epilepsy in children.

5 The present invention can be used to treat convulsive disorders such as epilepsy. That is, the pharmaceutical compositions of the invention display "anticonvulsant activity," which is evidenced by a reduction of the severity, number, or duration of convulsions in animal models of epilepsy. Accordingly, the inventive pharmaceutical compositions should be useful in treating
10 conditions such as but not limited to simple partial seizures, complex partial seizures, status epilepticus, and trauma-induced seizures, as occur following head injury or surgery.

15 Epilepsy is a common disorder which has many causes, and it can be very difficult to control, often requiring treatment for many years to keep seizures under control. "At this time, there is no satisfactory treatment for epilepsy in a substantial proportion of patients. Clinical trials have shown that certain patients have a
20 better response to one drug than another, even when the patients have similar types of seizures and the drugs have similar mechanisms of action. The frequency and severity of side effects also varies substantially. Thus, multiple medications with different mechanisms of
25 action and attendant side effects will be needed for treatment of epilepsy until either epilepsy can be cured or a potent, safe new drug with broad activity is discovered" and developed. Dichter et al. (1996), *supra*.

30 **AFFECTIVE MOOD DISORDERS:** Under this rubric are conditions ranging from depression to dysphoric mania, i.e., mania, schizoaffective disorder, traumatic brain injury-induced aggression, post-traumatic stress disorder, panic states, and behavioral dyscontrol syndromes. Affective mood disorders have been treated
35 primarily prophylactically, with lithium salts, since the 1950s in Europe and since the 1970s in the United States.

Emrich et al., *J. Affective Disorders* 8: 243-50 (1985). In recent years, alternatives to lithium treatment have been under development, given several problems with lithium therapy. Newer, alternative therapies to lithium for affective mood disorders are therapies with anticonvulsants such as carbamazepine, benzodiazepines, valpromide, and valproate. Bernasconi et al., in *ANTICONVULSANTS IN AFFECTIVE DISORDERS*, pages 14-32 (Excerpta Medica 1984). Valproate has a lower propensity towards depressed arousal and mentation, memory impairment, and cognitive blunting than is seen with the benzodiazepines.

Despite the demonstrated efficacy of valproate in a multitude of affective disorders, the hepatotoxicity, mutagenicity, and gastric upset observed with its administration highlights the need for new therapeutic agents and treatments with improved side effect profiles. A pharmaceutical formulation according to the present invention is effective to this end, especially with respect to improved side effects. Notwithstanding any perceived structural similarity between, for example, valpromide and isovaleramide (see Figure 1), an absence is expected of valproate-related side effects which otherwise might detract from the efficacy of the present invention in treating the range of affective mood disorders.

CENTRAL NEUROPATHIC PAIN SYNDROMES: Conditions in this category, involving "neuropathic pain," affect a significant number of patients suffering from disorders of the brain or spinal cord, such as stroke, trauma, multiple sclerosis, and diabetes. Casey, in *PAIN AND CENTRAL NERVOUS SYSTEM DISEASE* (Raven 1991). Many GABAergic compounds are efficacious in various analgesia models relevant to identifying therapeutic candidates for treating neuropathic pain. See Lloyd & Morselli, in *PSYCHOPHARMACOLOGY: THE THIRD GENERATION OF PROGRESS*

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(Raven Press 1987). In a related vein, the use of anticonvulsants like valproate to treat various pain states has been documented extensively. Swendlow, J. *Clin. Neuropharmacol.* 7: 51-82 (1984). Thus, a pharmaceutical formulation of the present invention can be applied in similar fashion to ameliorate neuropathic pain.

HEADACHES: Headaches of the migraine type (Hering & Kuritzky, *Cephalalgia* 12: 81-84 (1992)), the cluster type (Hering & Kuritzky, *loc. cit.* 9: 195-98 (1989)) and the chronic type (Mathew & Sabiha, *Headache* 31: 71-74 (1991)) have been treated by the administration of valproate. Interactions with the GABAergic system are thought to play a major role in the etiology of these headaches and in the associated valproate therapy. For this reason, the present invention can alleviate symptoms associated with each of the three headache types, without the adverse side effects of valproate therapy.

RESTLESSNESS SYNDROME: The phrase "restlessness syndrome" denotes a somatic (non-mental) restlessness characterized by involuntary movement of the limbs, as well as by a sense of physical (rather than mental) agitation, which is independent of mood and, hence, is distinguished from restlessness *per se*. See Sachdev et al., *Austral. New Zealand J. Psychiatry* 30: 38-53 (1996).

The genus of restlessness syndromes, inclusive of numerous indications, can be observed in association with many organic and non-organic psychiatric illnesses. For example, drug-induced restlessness (tardive, chronic, and withdrawal akathisia), such as drug-induced extrapyramidal symptoms, is one of the most common side effects of neuroleptic drug therapy. Also within the restlessness-syndrome rubric are the so-called "restless leg syndrome" and "sleep-related periodic leg movements," pathologies that can be associated with head and/or spinal cord trauma and with lesions of the spinal cord.

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Idiopathic restless leg syndrome follows an autosomal dominant inheritance, with a variable clinical expression of symptoms.

Diminished GABAergic neurotransmission is implicated in the neurochemical basis of restlessness syndromes. Consistent with this notion, for instance, is the efficacy of the benzodiazepines, baclofen, valproate, and gabapentin in the treatment of restless leg syndrome, an important indication. See O'Keefe, *Arch. Intern. Med.* 156: 243-48 (1996); Danek et al., in *NEUROLOGICAL DISORDERS: COURSE AND TREATMENT*, pages 819-23 (Academic Press 1996); Mellick & Mellick, *Neurology* 45(suppl): 285-86 (1995). More generally, the present invention provides an effective therapy for restlessness syndromes with minimal side effects.

MOVEMENT DISORDERS: Various GABAergic agents are known to decrease the dyskinetic movement characterizing movement disorders such as Parkinson's disease, Huntington's chorea, tardive dyskinesia, and stiff-man syndrome. This fact has highlighted a role for central GABAergic function in the control and modulation of CNS excitability and movement. Lloyd & Morselli (1987), *supra*. By the same token, a therapy within the present invention can effect a reduced level of CNS activity, presumably via a GABAergic mechanism, to alleviate one or more symptoms of a movement disorder.

3. METHODS FOR PREPARING PHARMACEUTICAL FORMULATIONS

The rhizomes and roots of *Valeriana* spp. (common name: valerian; family Valerianaceae) have been used for medicinal purposes since ancient times. The most commonly used valerian preparations include aqueous and hydroalcoholic extracts, such as tinctures, intended for oral administration. In addition, ammoniated valerian tinctures were used medicinally in the English-speaking world since at least the beginning of the seventeenth

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century. Hobbs, *HerbalGram* No. 21: 19-34 (1989). In the last three decades, the sedative and antispasmodic properties of valerian preparations have been attributed primarily to the presence of chemically labile monoterpenoid iridoid triester compounds called valepotriates ("valerian-epoxy-triesters (-ates)").

The most common and abundant of the valepotriates, valtrate and didrovaltrate, each contain two isovalerate moieties esterified to a "central" iridoid nucleus. Lin et al., *Pharm. Res.* 8: 1094-02 (1991). These acid- and heat-labile substances do not survive intact in the stomach following oral administration, however, readily releasing two moles of isovaleric acid for every mole of valepotriate. Furthermore, aqueous extracts of valerian rhizomes and roots retain their biological properties, even though the valepotriate triesters are water-insoluble. Bos et al., *Phytochem. Anal.* 7: 143-51 (1996).

The major, water-soluble, active principle of commonly used valerian extracts and other preparations, such as aqueous or hydroalcoholic extracts or tinctures, has been determined to be the ester hydrolysis product, isovaleric acid. Ammonium isovalerate and isovaleramide are produced in ammoniated tinctures. Balandrin et al., *J. Toxicol.-Toxin Rev.* 14: 165 (1995). The structures of isovaleramide and related compounds are depicted in Figure 1. In this way, the chemically labile valepotriates and other valerian-derived monoterpenoid-isovalerate esters, such as bornyl, lavandulyl, and ethyl isovalerates, act as "pro-drugs" and chemical precursors for isovaleric acid, its salts, and isovaleramide.

Isovaleramide has been isolated from valerian plants, most probably as an isolation artifact following treatment with ammonia. Buckova et al., *Cesk. Farm.* 26: 308 (1977); *Chem. Abstr.* 88: 86063z (1978); see also Bos et al. and Fuzzati et al., *Phytochem. Anal.* 7: 143, 76

(1996). More Recently, isovaleramide was shown to exhibit low acute toxicity *in vivo*, no mutagenic potential, and clinically useful anxiolytic properties. U.S. patent No. 5,506,268; PCT application WO 94/28,888. Methods for preparing isovaleramide are well known.

Extracts of medicinal plants that are useful for treating the symptoms of spasticity and convulsions can be prepared by aqueous, hydroalcoholic, or alcoholic extraction, or by extraction with other suitable solvents using methods well known to those of skill in the art. In the context of the present invention, useful extracts contain at least one of the following: isovaleric acid, its salts or complexes, ethyl isovalerate, isovaleramide, N-ethyl isovaleramide, and their chemical precursors. Useful extracts also share the common property of releasing isovaleric acid and/or isovaleramide upon hydrolysis *in vivo*. Standard methods for preparing such extracts can be found in pre-1950 editions of the U.S. PHARMACOPOEIA (U.S.P.) and the NATIONAL FORMULARY (N.F.), as well as in well-known references such as Gennaro (Ed.), REMINGTON'S PHARMACEUTICAL SCIENCES, 18th ed. (Mack Publishing Co. 1990), Tyler et al., PHARMACOGNOSY, 9th ed. (Lea and Febiger 1988), and Hare et al., THE NATIONAL STANDARD DISPENSATORY (Lea Brothers 1905). Additional citations appear in U.S. patent No. 5,506,268 and PCT application WO 94/28,888.

The principal historic sources of naturally occurring isovaleric acid have been valerian rhizomes and roots, as well as those of closely related plants in the family Valerianaceae. As discussed by Hobbs (1989), *supra*, these include the common valerian plant, *Valeriana officinalis* L., as well as the East Indian valerian, *V. wallichii* DC. and the biblical spikenard, *Nardostachys jatamansi* (Roxb.) DC. In addition to valerian rhizomes and roots, other plants which have been used traditionally as sedative or "antispasmodic" herbal

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medicines are known to contain, or to produce, isovaleric acid. These include hops (*Humulus lupulus* L., family Moraceae, which is often used in herbal formulations in combination with valerian), "cramp bark" or "guelder rose" (*Viburnum opulus* L., family Caprifoliaceae), and "black haw" (*V. prunifolium* L., root bark). Hare et al., THE NATIONAL STANDARD DISPENSATORY, pages 93, 94, 159, 160, 169, 256, 642, 692-694, 766, 767, 932, 1031, 1383, 1384, 1426, 1479, 1480, 1571, 1572, 1619, 1620, 1631-1633, 1661, and 1662 (Lea Brothers 1905); Heyl et al., J. Am. Chem. Soc. 42: 1744 (1920); Grier, Pharm. J. Pharm. 68: 302 (1929); Grier, Chem. Drug. (London) 110: 420 (1929); Grieve, A MODERN HERBAL, pages 35-40, 265-276, 381, 382, 411-415, 744-746, 781, 782, and 824-830 (Hafner 1959); Holbert, J. Am. Pharm. Assoc., Sci. Ed. 35: 315 (1946); Hoffmann, THE HERBAL HANDBOOK: A USER'S GUIDE TO MEDICAL HERBALISM, pages 38, 39, 83 and 84 (Healing Arts Press 1989).

As in the case of valerian rhizomes and roots, hops generate isovaleric acid from more chemically complex precursors upon oxidation or enzymatic breakdown. Millspaugh, AMERICAN MEDICINAL PLANTS, AN ILLUSTRATED AND DESCRIPTIVE GUIDE TO THE AMERICAN PLANTS USED AS HOMEOPATHIC REMEDIES, pages 622-626 (Dover 1974); Hare et al., THE NATIONAL STANDARD DISPENSATORY, pages 766-767 (Lea Brothers 1905); Grier, Chem. Drug. (London) 110: 420 (1929); Grieve, A MODERN HERBAL, pages 411-415 (Hafner 1959); Stevens, Chem. Rev. 67: 19 (1967); Duke, CRC HANDBOOK OF MEDICINAL HERBS, page 557 (CRC Press 1985).

Pharmaceutically acceptable salts of organic acids, such as isovaleric acid, which have been approved by the U.S. Food and Drug Administration for commercial marketing include sodium, potassium, lithium, zinc, aluminum, calcium, or magnesium salts. REMINGTON'S PHARMACEUTICAL SCIENCES, 18th ed., page 1445 (Mack

Publishing Co. 1990). Salts of isovaleric acid that are commercially available in the United States include the ammonium, sodium, potassium, and zinc isovalerates.

Pharmaceutically acceptable alcohols can form esters with isovaleric acid via the corresponding isovaleric acid chloride and/or anhydride by methods that are well known in the art. See, for example, March, ADVANCED ORGANIC CHEMISTRY: REACTIONS, MECHANISMS, AND STRUCTURE, fourth ed. (John Wiley and Sons 1992). Such alcohols contain at least one hydroxyl or phenol moiety, and are well tolerated *in vivo*. Examples of suitable alcohols include ethanol, certain carbohydrates and related compounds such as glucose, fructose, sucrose, xylose, and lactose, sugar alcohols such as dulcitol, mannitol, and sorbitol, sugar acids such as gluconic and glucuronic acids, glycerol, the polyol inositol, benzyl alcohol, certain phenols such as phenol, salicylic acid, saligenin, salicylamide, vanillin, *p*-hydroxycinnamic acid (*p*-coumaric acid), caffeic acid, ferulic acid, gallic acid, ellagic acid, quercetin, and eugenol. Other examples of suitable alcohols include alkaloids and biogenic amines such as ephedrine, pseudoephedrine, phenylpropanolamine, tyramine, and dopamine, vitamins such as ascorbic acid (vitamin C), thiamine (vitamin B1), riboflavin (vitamin B2), pyridoxine (vitamin B6), cyanocobalamin (vitamin B12), the tocopherols (vitamin E), choline, folic acid, and pantothenic acid, monoterpene alcohols such as geraniol, nerol, and linalool, naturally occurring triterpene alcohols such as α - and β -amyriins, lupeol, and oleanolic and ursolic acids, bile acids such as cholic acid, deoxycholic acid, and taurocholic acid, and common naturally occurring plant sterols (phytosterols) such as β -sitosterol, stigmasterol, campesterol, and brassicasterol. Tyler et al., PHARMACOGNOSY, 9th ed. (Lea and Febiger 1988). Other such well-tolerated hydroxyl- and phenol-containing

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compounds can be readily identified by those skilled in the art by consulting standard reference works such as THE MERCK INDEX and REMINGTON'S PHARMACEUTICAL SCIENCES, 18th ed. (Mack Publishing Co. 1990). Esters of isovaleric acid that are commercially available in the United States include the bornyl, ethyl, n-butyl, isoamyl, and geranyl isovalerates.

Isovaleric acid, ammonium isovalerate, and the esters ethyl isovalerate, isoamyl isovalerate, 2-methylbutyl isovalerate, cinnamyl isovalerate, methyl isovalerate, bornyl isovalerate, isobornyl isovalerate, and menthyl isovalerate, among other isovalerate esters, are listed in the Code of Federal Regulations by the FDA as being acceptable flavoring agents which may be used in foods. 21 CFR §172.515 (1991). Valerian (*Valeriana officinalis* L.) rhizomes and roots and black haw (*Viburnum prunifolium* L.) bark are listed as acceptable natural flavoring substances and natural adjuvants in 21 CFR §172.510 (1991). Hops and "lupulin" are listed among substances that are generally considered to be safe ("GRAS"). 21 CFR §182.20 (1991).

Generally, esters of isovaleric acid are expected to be hydrolyzed *in vivo* by ubiquitous esterase enzymes, thereby releasing isovaleric acid and the constituent alcohol or phenol. Particularly preferred among the isovalerate esters are glyceryl mono-, di-, and especially tri-isovalerates ("triisovalerin"), isovaleryl salicylic acid or salicylate (salicylic acid isovalerate), ethyl isovalerate, and β -sitosteryl isovalerate. See Figure 1. Hydrolysis of these isovalerate esters *in vivo* releases isovaleric acid and glycerol (glycerin), salicylic acid (an analgesic, anti-inflammatory, and febrifuge), ethanol (ethyl alcohol or common "alcohol," a CNS depressant), and β -sitosterol (a harmless phytosterol), respectively. With the exception of ethyl isovalerate, these esters are non-volatile or

only slightly volatile, thereby minimizing any unpleasant odors. Furthermore, in pure form these esters possess the advantage of having neutral to pleasant odors, in contrast to the extremely unpleasant odors of isovaleric acid and its salts, such as the ammonium, sodium, potassium, and zinc isovalerate salts. Moreover, whereas ethyl isovalerate is a liquid, the glyceryl mono-, di-, and tri-isovalerates, isovaleryl salicylate, and β -sitosteryl isovalerate are expected to be solids at room temperature, thereby facilitating their formulation into various standard solid and liquid oral dosage forms well known in the art, such as tablets (e.g., uncoated tablets, enteric-coated tablets, and film-coated tablets), capsules, gelcaps, powders, concentrates (drops), elixirs, tinctures, and syrups.

In addition to isovaleramide, various substituted amides of isovaleric acid can be prepared by methods well known in the art. See, for example, March, ADVANCED ORGANIC CHEMISTRY: REACTIONS, MECHANISMS, AND STRUCTURE, 4th ed. (John Wiley and Sons 1992). Preferred amides include N-ethyl isovaleramide, N-methyl isovaleramide, N,N-dimethyl isovaleramide, N-methyl, N-ethyl isovaleramide, N-isovaleryl GABA, and N-isovaleryl glycine. See, for example, Tanaka et al., *J. Biol. Chem.* 242: 2966 (1967). N,N-Diethyl isovaleramide ("Valyl"), although purported to possess CNS depressant (sedative) activity, has recently been shown to possess CNS stimulant (convulsant) properties; see U.S. patent No. 5,506,268 and PCT application WO 94/28,888, *supra*. An amide of isovaleric acid with p-aminophenol also can be prepared using standard methods to provide a compound, "isovaleraminophen," which is related structurally to the drug acetaminophen (Tylenol®; see Figure 1). In a manner analogous to that of the isovalerate esters, these substituted amides should be hydrolyzed *in vivo* (in this

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case, via hepatic amidase enzymes), releasing isovaleramide or isovaleric acid.

5 The compounds and preparations discussed above represent alternative forms for delivering isovaleric acid or isovaleramide *in vivo*. In certain cases, such as
10 with isovaleryl salicylic acid and ethyl isovalerate, the pharmacologically active moiety corresponding to the alcohol or phenol portion may be expected to exert its own pharmacological effects. For example, compounds such
15 as "isovaleraminophen" would be expected to exhibit a "Tylenol®-like" effect, as well as the effect expected from the isovaleric acid or isovaleramide moiety. Such novel chemical combinations of a previously known, pharmacologically active alcohol, phenol, or primary or secondary amine with isovaleric acid fall within the scope of the present invention.

The pharmaceutical formulations of the present invention can be prepared according to known methods to prepare pharmaceutically useful compositions, whereby
20 active agents are combined in a mixture with a pharmaceutically acceptable carrier. For instance, see REMINGTON'S PHARMACEUTICAL SCIENCES and GOODMAN AND GILMAN'S, both cited above. A composition is said to be in a "pharmaceutically acceptable carrier" if its
25 administration can be tolerated by a recipient patient. Sterile phosphate-buffered saline is one example of a pharmaceutically acceptable carrier. Other suitable carriers (e.g. saline and Ringer's solutions) are well known to those skilled in the art. See, for example,
30 REMINGTON'S PHARMACEUTICAL SCIENCES, *supra*.

In general, the dosages of the antispasticity and anticonvulsant agents described herein will vary depending upon such factors as the patient's age, weight, height, sex, general medical condition, and previous
35 medical history. For purposes of therapy, a compound of the present invention and a pharmaceutically acceptable

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5 carrier are administered to a subject in need of such
treatment in a therapeutically effective amount. The
combination of active agent and carrier is said to be
administered in a "therapeutically effective amount" if
the amount administered is physiologically significant.
An agent is physiologically significant if its presence
results in a detectable change in the physiology of a
recipient patient. In the present context, for example,
10 an antispasticity agent is physiologically significant if
the presence of the agent results in the alleviation of
spasticity, while an anticonvulsant agent is
physiologically significant if the presence of the agent
results in the reduction of the severity, number, or
duration of convulsions.

15 Isovaleramide and related compounds can be
administered orally using solid oral dosage forms such as
enteric-coated tablets, caplets, gelcaps, or capsules, or
via liquid oral dosage forms such as syrups or elixirs.
The indicated dosage of isovaleramide and related
20 compounds as antispasticity agents is on the order of
100-1000 mg per dose, and preferably, 300-600 mg per
dose. Unit solid oral dosage forms preferably contain
about 200-350 mg per tablet or capsule, which typically
would be taken 1-2 at a time for a maximum of four times
25 per day, at a dosage of 1-20 mg/kg body weight. Liquid
formulations can also be employed with active ingredient
compositions so as to provide 1-2 teaspoonfuls per dose.
Furthermore, corresponding reduced dosage pediatric
chewable and liquid oral dosage forms can also be
30 administered. These compounds also can be added to foods
and beverages in the form of drops (with a dropper from
a "concentrate" preparation) for oral administration. In
addition, compounds such as isovaleramide may be
formulated into chewing gum to facilitate oral delivery
35 and absorption.

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Alternatively, isovaleramide and related compounds can be administered by injection or other systemic routes, such as transdermal or transmucosal administration, for example, nasally, buccally, or rectally, via suppositories. Oral administration is much more convenient, however, and therefore is preferred.

For use in an oral anticonvulsant composition, the dosage level of active ingredient(s) is on the order of 100-1000 mg per dose, and preferably, 200-600 mg per dose, or 1-20 mg/kg body weight.

In addition to a use in humans, isovaleramide and related compounds can be used, for example, as antispasticity agents or anticonvulsant agents, in animals such as cats, dogs, birds, horses, cattle, mink, poultry, and fish. In such cases the active compound may be administered by injection or other systemic routes, such as transdermal or transmucosal administration (for example, rectal administration via suppositories), or orally by addition to food or drink. As an antispasticity agent, the indicated oral dosage of isovaleramide and/or related compounds per kilogram of body weight of such animals is about 1-1000 mg/kg, depending upon the species of animal and the route of administration. A preferred range for oral dosage is about 200-600 mg/kg body weight.

The indicated oral dosage of isovaleramide and/or related compounds per kilogram body weight as anticonvulsant agents for animals is in the range of about 1-1000 mg/kg, depending upon the species of animal and the route of administration. A preferred range for oral dosage is about 100-600 mg/kg body weight.

The present invention thus contemplates a variety of pharmaceutical compositions containing isovaleramide, isovaleric acid, and/or its pharmaceutically acceptable salts, substituted amides, and alcohol esters as active ingredients that are suitable for oral, parenteral,

transdermal, transmucosal, intranasal, buccal, or rectal administration. Although such compounds may be present as incidental by-products in certain pharmaceutical formulations which are outside the scope of the present invention, the common feature of the present formulations is that isovaleramide, isovaleric acid, and/or its pharmaceutically acceptable salts, substituted amides, and alcohol esters is present in a standardized amount. That is, the pharmaceutical formulations contain a predetermined, chemically defined, and quantifiable amount of at least one of such compounds to enable the determination of the quantity of a particular composition required to achieve the dosage levels described herein.

It is further understood that isovaleramide and/or related compounds can be used in combination with other pharmaceutically active ingredients.

4. DEMONSTRATING THERAPY-IMPLICATING ACTIVITY

The suitability and effectiveness of a given pharmaceutical formulation for the alleviation of a pathology, as discussed above, can be demonstrated using animal models such as (but not limited to) those described below.

(a) The Mutant Spastic Mouse

The mutant spastic mouse is a homozygous mouse that carries an autosomal recessive trait of genetic spasticity. The mouse is normal at birth, and then the mouse develops a coarse tremor, abnormal gait, skeletal muscle rigidity, and abnormal righting reflexes at two to three weeks of age. No structural abnormalities have been found. Rather, the mouse has a deficit of glycine receptors throughout the central nervous system. Drugs that either potentiate the binding or synthesis of GABA, such as valproate and the benzodiazepines, are effective compounds to ameliorate some of the symptoms of spasticity in this model, as well as in humans.

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5 The assessment of spasticity in the mutant spastic
mouse can be performed by electrophysiological assessment
similar to the EMG recordings described below. One can
also, on a more crude scale, measure righting. These
10 mice have an abnormal delayed righting reflex when placed
on their backs. Any righting reflex over one second is
considered abnormal. Most normal mice cannot even be
placed on their backs. Tremor can be evaluated by
holding mice by their tails and rating the tremor
15 subjectively by "absent," "slight," "moderate," or
"severe." Flexibility is assessed by placing the mouse
on a glass object with smoothly rounded grooves and rims.
The glass object is lifted about 12 inches above the
table and slowly tilted until almost vertical. Normal
20 mice will climb about the object for a minute or more
before falling to their feet. Spastic mice usually
remain stiffly in one position and soon fall onto their
backs. Chai et al., *Proc. Soc. Exptl. Biol. Med.* 109:
491 (1962).

20 (b) **The Acute/Chronic Spinally Transected Rat And
The Acute Decerebrate Rat**

There are several models of spasticity including the
acute decerebrate rat, the acute or chronic spinally
transected rat, and the chronically spinal cord-lesioned
25 rat. The acute models, although of proven value in
elucidating the mechanisms involved in the development of
spasticity, have come under criticism due to the fact
that they are acute. The animals usually die or have
total recovery from spasticity. The spasticity develops
30 immediately upon intervention, unlike the spasticity that
evolves in the human condition of spasticity, which most
often initially manifests itself as a flaccid paralysis.
Only after weeks and months does spasticity develop in
humans. Some of the more chronic-lesioned or spinally
35 transected models of spasticity do post-operatively show
flaccid paralysis. At approximately four weeks post-

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5 lesion/transection, the flaccidity changes to spasticity
of variable severity. Although all of these models have
their own particular disadvantages and lack of true
representation of the human spastic condition, they have
provided much information about the nature of spasticity.
These models have also provided methods to test various
treatment paradigms that have led to similar treatments
being tested in humans. Many of these models have also
made use of different species, such as cats, dogs, and
10 primates. Baclofen, diazepam, and tizanidine are
effective on different parameters of spasticity (EMG
recordings, H-reflex, the H/M ratio, mono- and
polysynaptic reflexes, clonus, hyperreflexia) in these
models.

15 (c) Primary Observation Irwin Test In The Rat

This method is based on that described by Irwin,
Psychopharmacologia 13: 222-57 (1968). It is used to
detect physiological, behavioral, and toxic effects of a
test substance, and indicates a range of dosages that can
20 be used for later experiments. Typically, rats (three
per group) are administered the test substance and are
then observed in comparison with a control group given
vehicle. Behavioral modifications, symptoms of
neurotoxicity, pupil diameter, and rectal temperature are
25 recorded according to a standardized observation grid
derived from that of Irwin. The grid contains the
following items: mortality, sedation, excitation,
aggressiveness, Straub tail, writhes, convulsions,
tremor, exophthalmos, salivation, lacrimation,
30 piloerection, defecation, fear, traction, reactivity to
touch, loss of righting reflexes, sleep, motor
incoordination, muscle tone, stereotypies, head-weaving,
catalepsy, grasping, ptosis, respiration, corneal reflex,
analgesia, abnormal gait, forepaw treading, loss of
35 balance, head twitches, rectal temperature, and pupil
diameter. Observations are performed at 15, 30, 60, 120,

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and 180 minutes following administration of the test substance, and also 24 hours later. The test substance is usually administered intraperitoneally (i.p.).

(d) Rotarod Test In The Rat and Mouse

5 This is a test of neurological deficits using the method described by Dunham et al., *J. Am. Pharm. Assoc.* 46: 208-09 (1957). Rats or mice are placed on a rod rotating at a speed of eight turns per minute. The number of animals which drop off the rod before three
10 minutes is counted and the drop-off times are recorded (maximum: 180 sec). Ten rats are studied per group and the test is performed blind. The test compound is administered i.p. 60 min prior to testing. Diazepam, a benzodiazepine, is administered at 8 mg/kg, i.p., as the
15 reference substance. A control group administered the vehicle is also included in the study.

(e) Anticonvulsant Activity

There are numerous *in vivo* models involving different kinds of seizures and behavioral effects that are
20 relevant for clinically distinct forms of epilepsy. It therefore is prudent to test for effects in several models, because it may be an oversimplification to suppose that the same mechanism underlies all forms of seizure activity.

25 One useful model is provided by the Frings audiogenic seizure-susceptible mouse, a model of reflex epilepsy. At the time of testing, individual mice are placed into a round Plexiglas chamber and exposed to a sound stimulus of 110 decibels, 11 kHz, for 20 seconds. Animals not
30 displaying tonic hindlimb extensions were considered protected. In addition, the seizure score for each mouse can be recorded as: (1) running for less than 10 seconds; (2) running for greater than 10 seconds; (3) clonic activity of limbs and/or vibrissae; (4) forelimb
35 extension/hindlimb flexion; and (5) hindlimb extension.

5 The average seizure score can be calculated for each group of mice employed in the dose-response study. At each dose, mice are also tested on a rotarod for testing of motor impairment (toxicity). Testing for motor impairment on the rotarod involves placing a mouse for a three-minute trial period on a one-inch diameter rod rotating at six revolutions per minute. If the mouse falls off of the rotating rod three times within the three-minute time period, it is considered a toxic response.

10 (f) **Anti-Manic Activity**

To assess the possible use of compounds in the treatment of affective mood disorders, one can employ the amphetamine-induced hyperactivity model in rats. In addition to being a test for classical and atypical antipsychotic activity, this procedure has also been proposed as a simple animal model of manic behavior. Costall et al., *Brain Res.* 123: 89-111 (1977).

15 (g) **Neurogenic Inflammation Of The Meninges**

20 Neurogenic inflammation within the meninges has been proposed as an event in the underlying pathology of migraine headaches. Lee et al., *Brit. J. Pharmacol.* 116: 1661-67 (1995). Compounds are tested for their ability to block the leakage of radiolabeled bovine serum albumin within the dura mater post trigeminal stimulation.

25 (h) **Analgesic Properties**

30 There are many whole-animal assays for determining analgesic properties, such as writhing, hotplate, tail flick, arthritic pain, paw pressure tests, and the Bennet or Chung models of neuropathic pain. Albe-Fessard et al., in 13 *ADVANCES IN PAIN RESEARCH AND THERAPY*, pages 11-27 (Raven Press 1990).

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(i) **Therapeutic Benefit Relative To Movement Disorders and Restlessness Syndromes**

Animal models exist for the study of movement disorders and restlessness syndromes, for example, drug-induced akathisia, serotonin syndrome, rotation induced by unilateral nigral lesions. Lloyd & Morselli (1987), *supra*. Additionally, individual case reports of anecdotal efficacy of compounds in humans have been a source for support for these indications. Mellick & Mellick (1995), *supra*; Olson et al., *Am. J. Med.* 102: 60-66 (1997).

The therapeutic effects of isovaleramide, isovaleric acid, and related compounds in various of the assays described above, combined with a general lack of toxicity, make the compounds of the present invention ideal agents for the treatment of the pathologies described above, including spasticity and convulsions/seizures. With this background, the present invention will be understood more readily by reference to the following examples, which are provided for purposes of illustration and are not intended to be limiting of the invention.

Example 1

Use of a Valerian Preparation to Alleviate Symptoms of Spasticity Associated with Multiple Sclerosis

A human female subject, age 42, suffering from one or more symptoms of multiple sclerosis, was experiencing a considerable amount of stress and was experiencing difficulty in getting to sleep and delayed onset of sleep at night. The sleep that did occur was disturbed by stressful dreams and frequent awakening as well. The subject also experienced frequent nighttime painful extensor spasms of the lower extremities that would often awaken the subject from her sleep. The following day,

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these painful extensor spasms resulted in a deep muscle pain (bruising sensation), with muscle/joint stiffness.

5 The subject decided to consume a preparation of
valerian that was noted for its sleep-aid properties.
The valerian product, "Baldriparan stark N," consists of
10 tablets manufactured in Germany that contain extracts of
valerian root, hops, and lemon balm. The coated, pressed
tablets each contain 95 milligrams of a dried 70% (v/v)
ethanol extract of valerian root, 15 milligrams of a
15 dried 45% (m/m) methanol extract of hops, and 85
milligrams of a dried water extract of lemon balm.
Surprisingly, the valerian preparation not only
facilitated the onset of sleep and improved the quality
of sleep for the subject, but it was also noticed that
20 the painful extensor spasms were alleviated. The subject
noted that upon awakening during the night to use the
bathroom, she did not experience the painful extensor
spasms upon getting out of bed nor the usual stiff-leg
sensation. The subject continues to consume the same
valerian product for the alleviation of these symptoms on
an as-needed basis (*prn* or *pro re nata*) and continues to
experience relief.

Example 2

25 Use of a Valerian Preparation to Alleviate Symptoms of Spasticity Associated with Spinal Cord Injury

30 A human male subject, age 38, suffers symptoms of
spasticity (hyperreflexia, tendon jerks, and extensor
spasms) that evolved from an earlier injury to the spinal
cord. All of these symptoms interrupt and decrease the
quality of sleep experienced by this individual. Upon
taking the same German-made preparation of valerian
described in Example 1, the subject noted considerable
improvement in the quality of sleep as well as a
significant reduction in night-time extensor spasms.
35 This subject continues to consume the preparation on an

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as-needed (prn) basis for the alleviation of the symptoms described above.

Example 3

Isovaleramide Antispasticity Tests

(1) Assessment of Spasticity in Chronic Spinally Transected Rats

In these studies, male albino Holtzman-derived rats (Harlan Sprague-Dawley Laboratories) weighing 270-530 grams were used as subjects. The animals were housed independently and had continuous access to food and water throughout the experiments. All procedures were reviewed and approved by the Institutional Animal Care and Use Committee. Animals were anesthetized using a mixture of isoflurane and oxygen at a flow rate of 4 liters/minute. The rats were then placed in a stereotaxic frame and anesthesia was maintained. An incision was made so that the paraspinal muscles could be retracted and a laminectomy performed between T6-T9. A one- to two-millimeter portion of the spinal cord was removed by evacuation and replaced with Gel foam to reduce bleeding, after which the incision was closed in layers.

Following the transection, rats were placed in a room in which the ambient temperature was raised to about 80°F with a space heater to maintain body temperature. On the following morning post-surgery, the hindquarters of the spinalized rats were bathed and their urine expressed manually by applying pressure to their bladders. Experiments were conducted between 21 and 28 days after surgery. For the first two weeks, these rats were given 0.25 ml of the antibiotic Sulfatrim Pediatric Suspension orally to prevent bladder infection. A commercial antibiotic cream was applied to any part of the skin that showed signs of decubitus lesions. Within approximately two weeks, all animals regained bladder control and were no longer given antibiotic treatment. Advokat, Brain

Res. 684: 8 (1995). Assessment of spasticity was performed before and after drug treatment such that each animal served as its own control.

Initial assessment of spasticity was performed by the subjective scoring method of rating the resulting spasticity response elicited with an innocuous stimulus, i.e., a metal probe, that was pressed against the lower abdomen at four specific sites. The spastic reaction was evaluated for each of the four trials using a scale ranging from zero (no spastic response in all four trials) to four (a maximum, tonic-clonic reaction elicited in all four trials). All spasticity scores, pre- and post-treatment, were transformed to indicate the percent spasticity such that a score of $0/4 = 0\%$, $1/4 = 25\%$, etc. These raw or normalized scores were analyzed with a one-way repeated measures ANOVA.

As shown in Figure 2, isovaleramide at a dose of 300 mg/kg, i.p., was efficacious at 15, 30, 60, and 120 minutes post-administration in reducing the spasticity scores (45-65%). By the next day, i.e., by 1440 minutes (24 hours), the spasticity scores had essentially returned to baseline values. No overt behavioral toxicity or motor impairment was observed at this dose. The rats were alert and able to grasp with their non-paralyzed front paws as were the control, untreated rats.

With reference to Figure 3, the polysynaptic flexor-reflex responses, to test stimuli which activate high-threshold afferents, were recorded as EMG activity from the ipsilateral hamstring muscle. Supramaximal electric shocks were applied to the hindpaw, and recording electrodes were placed in the biceps femoris semitendinosus muscle. Five sets of stimuli were made at each time point. The flexor reflex was recorded, in both the pre-drug and the post-drug periods, every 30 minutes once a stable baseline response was achieved. See Hao et al., *Eur. J. Pharmacol.* 191: 407 (1990).

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Thus, the responses were determined in spinalized rats by observing the flexor-reflex response (Figure 3) before treatment and at each of 30, 60, 90, and 120 minutes following administration of isovaleramide (300, 600, and 1200 mg/kg p.o.), baclofen (10 mg/kg s.c.) and vehicle (water, 12 ml/kg p.o.), respectively.

Isovaleramide at all doses was shown to reduce the magnitude of the flexor-reflex responses, at all time points in a chronic spinalized rat, with spasticity. In this model, no change in the H/M reflex was observed with either baclofen or isovaleramide.

In Figure 4, the responses from Figure 3 are converted to a total-area-under-the-curve format, covering the entire, two-hour measurement period. All drug-treated groups differed significantly from vehicle ($p < 0.05$), based on a one-way analysis of variance. Between the drug-treated groups, no differences were found in total reduction of the flexor reflex over the two-hour period (pairwise multiple comparison, Student-Newman-Keuls method).

(2) Primary Observation Irwin Test in the Rat

Administered i.p. in the rat, isovaleramide induced no changes from saline-injected controls at doses up to 256 mg/kg. At 512 mg/kg, slight sedation from 60 to 120 minutes, loss of traction (observed in only one of three rats) at 120 minutes, and decreased muscle tone from 60 to 120 minutes were observed. At 1024 mg/kg, marked sedation up to 30 minutes was observed, becoming moderate up to 120 minutes, then slight at 180 minutes. Decreased fear was also observed at this dose up to 30 minutes and in one of three rats up to 120 minutes. Decreased reactivity to touch up to 120 minutes, decreased muscle tone up to 180 minutes, slight hypothermia up to 120 minutes, and an abnormal gait (rolling) from 60 to 80 minutes were also observed at this dose. Loss of

-32-

grasping and loss of righting reflex occurred, in one of three rats, at 15 minutes at this dose.

(3) Rotarod Test in the Rat and the Frings Mouse

Isovaleramide, administered at doses of 128, 256, and 512 mg/kg (i.p.) 60 minutes before a test on the rotarod, did not significantly affect rotarod performance in the rat. See Table 1. In contrast, diazepam dose-dependently decreased rotarod performance.

Table 1

Effects of Isovaleramide and Diazepam in the Rotarod Test in the Rat

Dose of: Isovaleramide (mg/kg) ^a	Number ^b of Rats Falling	Drop-Off Time (sec)		
		Mean ± S.E.M.	t value	% change from control
0	5	135.5 ± 18.0	-	-
128	6	134.5 ± 20.7 ^c	0.036	-1%
256	7	98.4 ± 23.3 ^c	1.261	-27%
512	7	115.9 ± 20.5 ^c	0.717	-14%
Diazepam (mg/kg) ^a				
4	9	55.8 ± 20.6 ^d	2.909	-59%
8	10+ ^e	16.3 ± 6.4 ^f	6.222	-88%

^a Isovaleramide and diazepam were administered i.p. 60 minutes before the rotarod test.

^b Ten rats per group.

^c Not significant according to Student's t Test.

^d p<0.01 according to Student's t Test.

^e p<0.05 according to Fisher's Exact Test.

^f p<0.001 according to Student's t Test.

-33-

Isovaleramide, administered at doses up to 150 mg/kg (i.p.) 15 minutes before a test on the rotarod in the Frings mouse, did not affect performance significantly. In contrast, doses of 300 mg/kg, 600 mg/kg, and 1000 mg/kg (i.p.) decreased rotarod performance in 1/8, 4/8, and 8/8 of Frings mice tested, respectively.

Example 4

Anticonvulsant Activity in the Frings Audiogenic Seizure-Susceptible Mouse Model of Epilepsy

The results of Table 2 demonstrate isovaleramide anticonvulsant activity in this animal model of epilepsy. Isovaleramide also displayed a quick onset and a relatively short duration of action. Anticonvulsant activity was noted as early as 15 minutes, but decreased substantially by two hours. All quantitative studies therefore were conducted at 15 minutes. At this time point, the median effective dose (ED_{50}) for protection against tonic extension was 126 mg/kg, i.p. In addition, a dose-dependent reduction in seizure score was observed at this time point. At doses markedly higher than those providing anticonvulsant activity (>300 mg/kg), animals treated with isovaleramide displayed behavioral toxicity that was characterized by their inability to maintain their balance on the rotarod. No notable toxicity was observed at doses less than 300 mg/kg. The median toxic dose (TD_{50}) for rotarod impairment was 531 mg/kg, i.p. Thus, the calculated protective index (TD_{50}/ED_{50}) was about 4.2.

Therefore, despite the relatively low potency of isovaleramide in this model, it still displayed a relatively good separation between activity and toxicity. Isovaleramide thus had a surprising and unexpected efficacy, based on existing structure-activity relationships for amides and their corresponding acids, as an anticonvulsant in the Frings audiogenic seizure-

-34-

5 susceptible mouse model of reflex epilepsy. The activity
profile of isovaleramide is similar to that of the broad-
spectrum anticonvulsant, sodium valproate. Compounds
similar in structure to valproate as well as isovaleric
10 acid have been shown in previous literature to elevate
GABA levels throughout the CNS. It is this function,
primarily, that the anticonvulsant activity of valproate
is attributed to, although other mechanisms have been
suggested. Isovaleric acid, on the other hand, was found
15 to be inactive as an anticonvulsant, although it was
reported to elicit a slight increase in GABA levels in
the brains of mice. For example, see Löscher et al.,
Neuropharmacology 24: 427 (1985); Keane et al., *loc. cit.*
22: 875 (1983); Keane et al., *Pharmacol. Res. Commun.* 17:
547 (1985).

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Table 2

Effect of Isovaleramide on the Audiogenic Seizure
Susceptibility of Frings Mice Following Intraperitoneal
Administration

Dose of Isovaleramide (mg/kg, i.p.)	Seizure Score \pm S.E.M.	Number ^a Protected of Eight Mice Tested	Number ^a Showing Toxicity of Eight Mice Tested
75	4.4 \pm 0.6	1	0
112.5	4.0 \pm 0.6	2	0
150	2.0 \pm 0.6	6	0
300	1.0 \pm 0	8	1
600	-		4
1000	-		8
ED ₅₀ for protection: 126 mg/kg (98.8 - 168 ^b)			
TD ₅₀ : 531 mg/kg (372 - 711 ^b)			

^a Measured at 15 minutes.^b 95% confidence interval.

In general, the historical literature on the structure-activity relationships of anticonvulsant activity around compounds similar to valproate have taught away from simple, unsubstituted compounds such as isovaleramide. It is thus a surprising and unexpected observation that isovaleramide has demonstrated an efficacy profile similar to that of valproate in the Frings audiogenic seizure-susceptible mouse model and a similar separation of activity between efficacy and toxicity as measured by rotarod performance. These observations indicate that isovaleramide is an effective therapeutic agent as a broad-spectrum anticonvulsant. Isovaleramide is known for its relative lack of toxicity in mutagenicity and cytotoxicity tests. See U.S. patent No. 5,506,268 and PCT application WO 94/28,888. On the

other hand, valproate has long been noted for its hepatotoxicity-causing profile. For example, see Löscher et al., *Neuropharmacology* 24: 427 (1985).

5 Although the foregoing refers to particular preferred
embodiments, it will be understood that the present
invention is not so limited. It will occur to those of
ordinary skill in the art that various modifications may
10 be made to the disclosed embodiments and that such
modifications are intended to be within the scope of the
present invention, which is defined by the claims below.

15 All publications and patent applications mentioned
in this specification are indicative of the level of
skill of those in the art to which the invention
pertains. All publications and patent applications are
herein incorporated by reference to the same extent as if
each individual publication or patent application were
specifically and individually indicated to be
incorporated by reference in its entirety.

What Is Claimed Is:

1. Use of a compound selected from the group consisting of isovaleric acid, a pharmaceutically acceptable salt of isovaleric acid, a pharmaceutically acceptable ester of isovaleric acid, and a pharmaceutically acceptable amide of isovaleric acid in the preparation of a pharmaceutical formulation for use in a method of treating a pathology that is ameliorated by a mild depression of CNS activity, whereby at least one symptom of said pathology is alleviated.

2. A use according to claim 1, wherein said pathology is selected from the group consisting of an affective mood disorder, convulsions, a central neuropathic pain syndrome, a headache, and a restlessness syndrome.

3. A use according to claim 2, wherein said pathology is convulsions.

4. A use according to claim 1, wherein said pathology is ameliorated by a centrally mediated decrease in muscle tone.

5. A use according to claim 4, wherein pathology is spasticity.

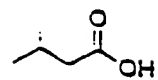
6. A use according to claim 1, wherein said composition is isovaleramide.

7. Use of an extract of Valerianaceae, cramp bark, black haw, or hops in the preparation of a pharmaceutical formulation for use in a method of treating a symptom of spasticity, wherein said extract comprises at least one compound that is hydrolyzed *in vivo* to yield isovaleric acid or isovaleramide.

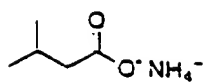
8. A treatment method comprising the step of administering, to a patient suffering from a pathology that is ameliorated by a mild depression of CNS activity, a therapeutically effective amount of a pharmaceutical formulation comprising a pharmaceutically acceptable carrier and a composition selected from the group consisting of isovaleric acid, a pharmaceutically acceptable salt of isovaleric acid, a pharmaceutically acceptable ester of isovaleric acid, and a pharmaceutically acceptable amide of isovaleric acid, whereby at least one symptom of said pathology is alleviated.

9. A method for alleviating a symptom of spasticity in a subject in need of such treatment, comprising the step of administering a therapeutically effective amount of an extract of Valerianaceae, cramp bark, black haw or hops, wherein said extract comprises at least one compound that is hydrolyzed *in vivo* to yield isovaleric acid or isovaleramide.

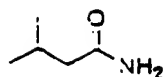
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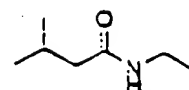
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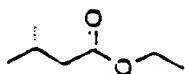
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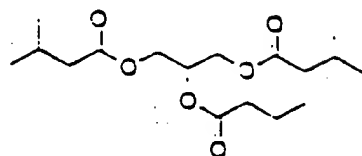
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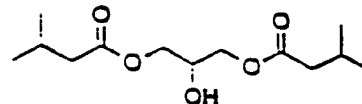
N-Ethyl isovaleramide



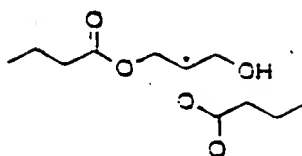
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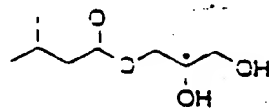
Glycerol triisovalerate ("Triisovalenn")



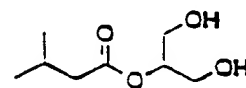
Glycerol 1,3-diisovalerate



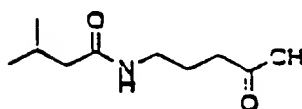
Glycerol 1,2-diisovalerate



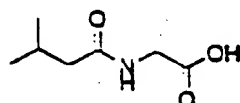
Glycerol 1-monoisovalerate



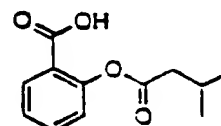
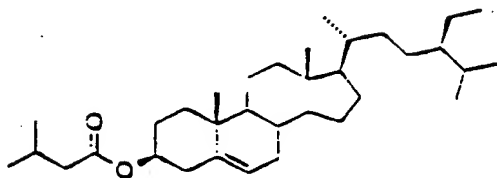
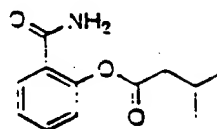
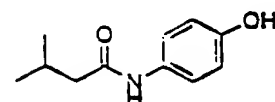
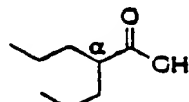
Glycerol 2-monoisovalerate



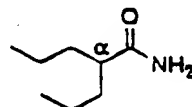
N-Isovaleryl GABA



N-Isovaleryl glycine

Isovaleryl salicylic acid
("I.S.A."); salicylic acid
isovalerate β -Sitosteryl isovalerateIsovaleryl salicylamide
(salicylamide isovalerate
ester)"Isovaleraminophen"
(N-(4-hydroxyphenyl)-
isovaleramide)

Valproic acid



Valpromide

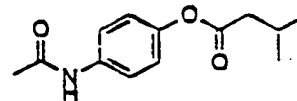
Isovaleryl-acetaminophen
ester

FIGURE 1.

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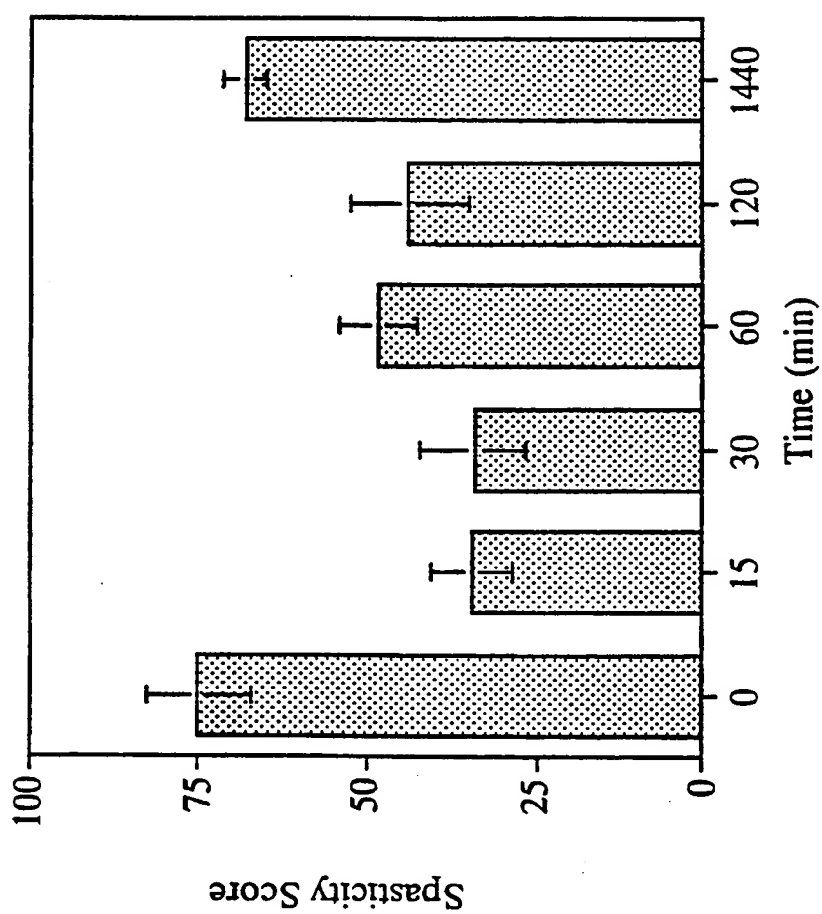


Figure 2.

The Flexor Reflex in the Chronic Spinalized Rat

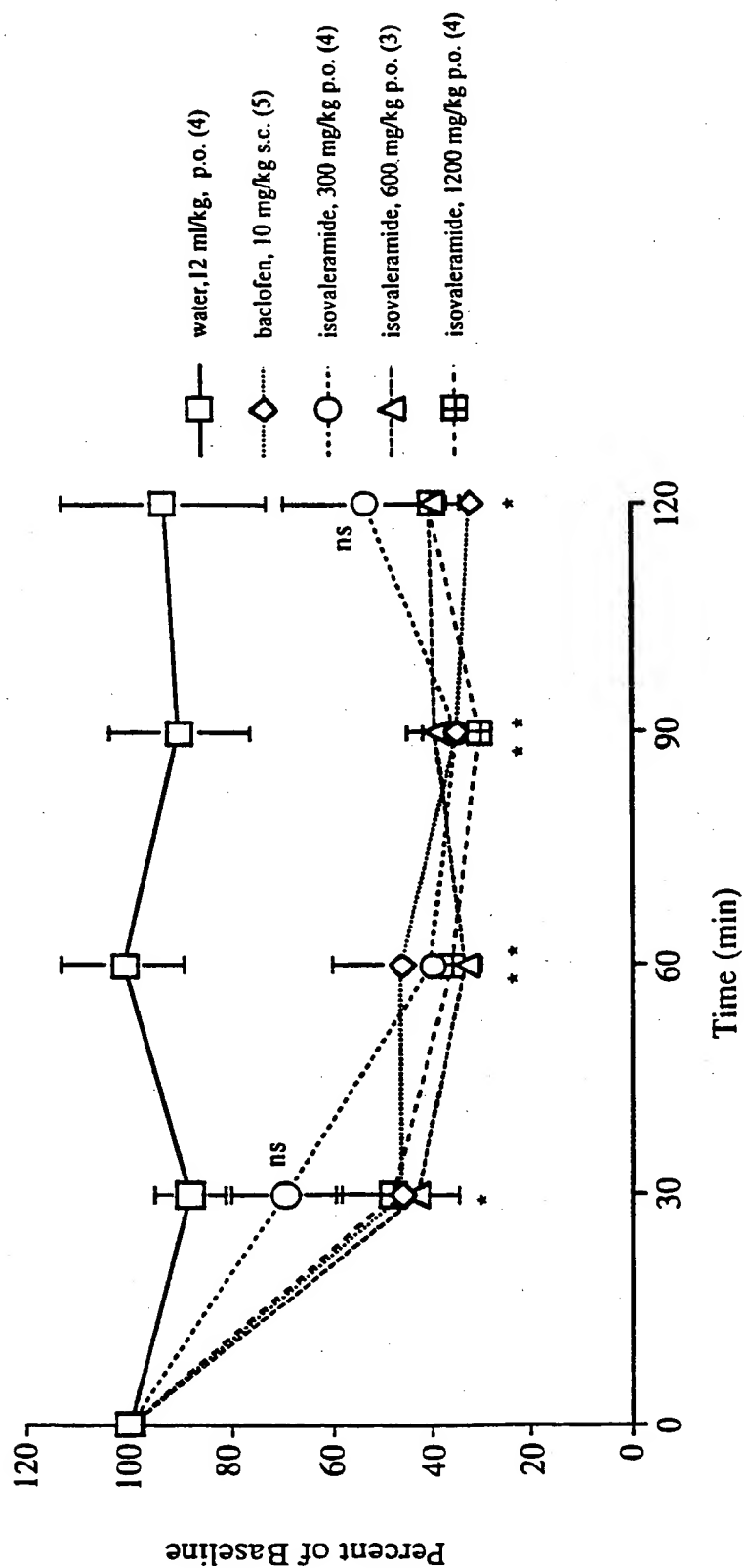


Figure 3.

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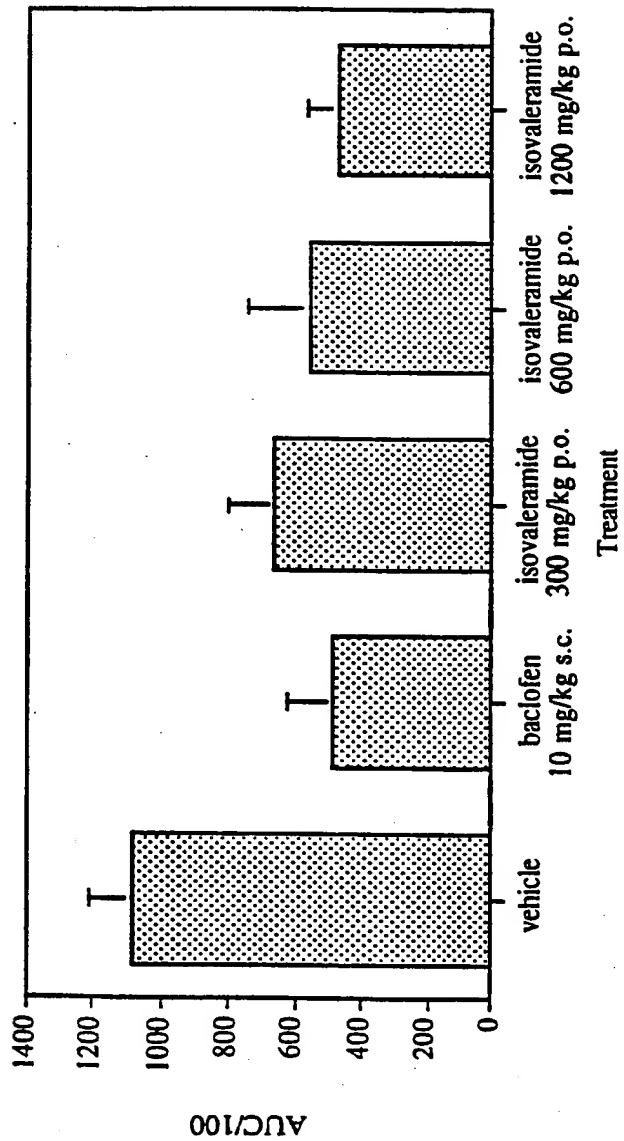


Figure 4.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 97/15272

A. CLASSIFICATION OF SUBJECT MATTER
 IPC 6 A61K31/16 A61K35/78

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KEANE ET AL.: "EFFECT OF VALPROATE ON BRAIN GABA : COMPARISON WITH VARIOUS MEDIUM CHAIN FATTY ACIDS" PHARMACOL. RES. COMMUN., vol. 17, no. 6, 1985, pages 547-555, XP002049841 in particular table 1 page 551 see the whole document ---	1-3
X	HAZELHOFF ET AL.: "Antispasmodic effects of Valeriana Compounds: An in-Vivo and in-Vitro Study on the Guinea-Pig Ileum" ARCH. INT. PHARMACODYN., vol. 257, no. 2, 1982, pages 274-287, XP002049842 see the whole document ---	1,4,7
	-/--	



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- "&" document member of the same patent family

Date of the actual completion of the international search

16 December 1997

Date of mailing of the international search report

21. 01. 98

Name and mailing address of the ISA

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Authorized officer

A. Jakobs

INTERNATIONAL SEARCH REPORT

Internat al Application No

PCT/US 97/15272

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MORAZZONI ET AL.: "Valeriana officinalis: traditional use and recent evaluation of activity" FITOTERAPIA, vol. 66, no. 2, 1995, pages 99-112, XP002049843 see the whole document ---	1,2,5, 7-9
X	BALANSARD ET AL.: "Critères de choix d'un extrait de Viburnum, Viburnum Prunifolium L., en fonction de ses actions toniveineuse et spasmolytique." PLANT MED. PHYTOTHER., vol. 17, no. 3, 1983, pages 123-132, XP002049844 see the whole document ---	7-9
X	FURNADJIEV G.: "PHYTOTHERAPEUTIC AND EXPERIMENTAL DATA ON SOME MEDICINAL PLANTS WITH SPASMOLYTIC EFFECT AND THEIR APPLICATION IN GASTROINTESTINAL DISEASES" PROBL. VATR. MED., vol. 8, no. 3, 1980, pages 79-86, XP002049845 see abstract ---	7-9
X	WAGNER ET AL.: "Ueber die spasmolytische Wirkung des Badrians" PLANTA MED., vol. 37, no. 1, 1979, pages 84-86, XP002049846 see the whole document ---	7-9
X	US 3 485 851 A (THIES PETER WILLIBRORD) 23 December 1969 see column 1, li 36-39 see the whole document ---	1-3,5, 7-9
X	DE 22 30 626 A (SCHAETTE ROLAND DR) 10 January 1974 see in particular page 1 see the whole document ---	7-9
X	WO 94 28888 A (NPS PHARMA INC ;BALANDRIN MANUEL F (US); WAGENEN BRADFORD C VAN (U) 22 December 1994 in particular pages 12-16 see the whole document ---	1,2,6-9

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INTERNATIONAL SEARCH REPORT

Internal Application No
PCT/US 97/15272

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>TAILLANDIER ET AL.: "Recherches dans la série dipropylacétique XII. Acides et alcools aliphatiques ramifiés anticonvulsivants"</p> <p>EUR. J. MED. CHEM., vol. 10, no. 5, 1975, POC038, pages 453-462, XP002049847 see in particular table 1, page 455 see the whole document</p>	1-3
X	<p>WEISS R.F.: "Herbal Medicine"</p> <p>1988 , AB ARCANUM , GOTHENBURG, SWEDEN XP002049848 00014 Chapter 11, pages 280-286, 290 and chapter 12 page 307, column 1.</p>	7-9
X	<p>BÉZANGER-BEAUQUESNE ET AL.: "Plantes médicinales des régions tempérées"</p> <p>1980 , MALOINE S.A. , PARIS XP002049849 00002 see pages 78, 363, 364 and 365</p>	7-9

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 97/15272

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
see FURTHER INFORMATION sheet PCT/ISA/210
2. ☒ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This international search report has not been established in respect of
-----the following reasons:

Claims Nos.: 1-4

because they relate to subject matter not required to be searched by this Authority, namely:

In view of the large number of compounds, which are defined by the general definition(s)/formulae used in claims 1-4, the search had to be restricted for economic reasons. The search was limited to the compounds for which pharmacological data was given and / or the compounds mentioned in the claims, and to the general idea underlying the application. (see Guidelines, chapter III, paragraph 2.3)

Claims Nos.: -

because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

In view of the large number of compounds, which are defined by the general definition(s)/formulae used in claims 1-4, the search had to be restricted for economic reasons. The search was limited to the compounds for which pharmacological data was given and / or the compounds mentioned in the claims, and to the general idea underlying the application. (see Guidelines, chapter III, paragraph 2.3)

INTERNATIONAL SEARCH REPORT

Information on patent family members

Internal Application No
PCT/US 97/15272

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 3485851 A	23-12-69	DE 1617555 A DK 115651 B NL 6705435 A	01-04-71 27-10-69 20-10-67
DE 2230626 A	10-01-74	NONE	
WO 9428888 A	22-12-94	US 5506268 A AU 7100694 A CN 1127472 A EP 0706385 A JP 9501908 T	09-04-96 03-01-95 24-07-96 17-04-96 25-02-97

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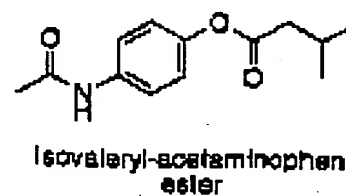
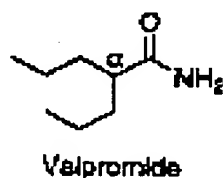
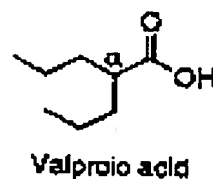
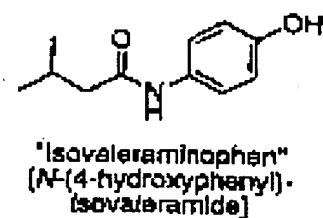
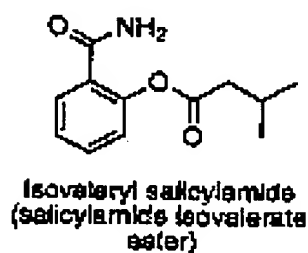
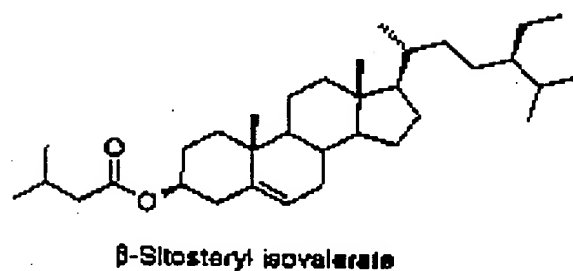
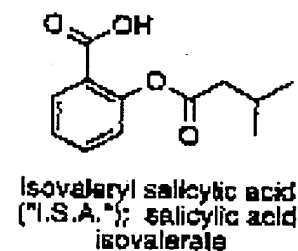
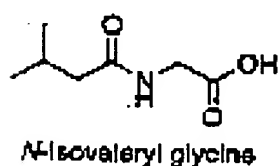
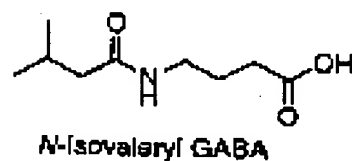
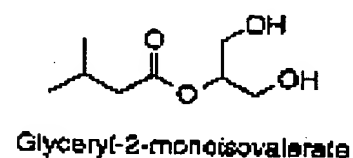
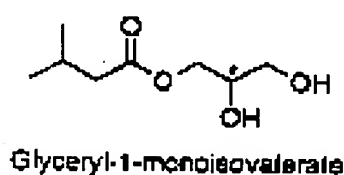
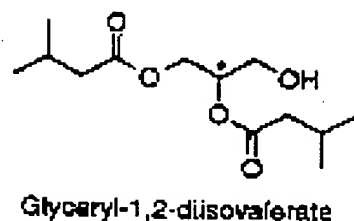
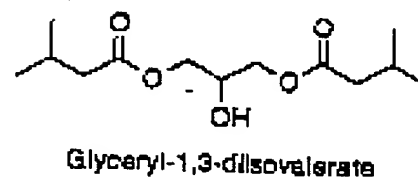
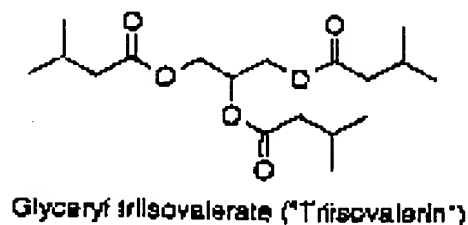
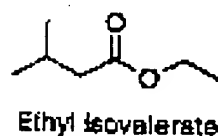
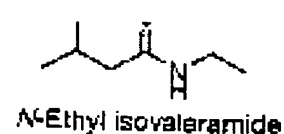
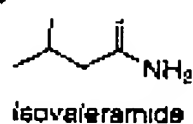
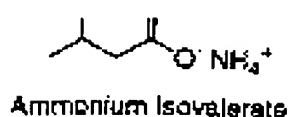
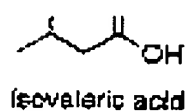


FIGURE 1.

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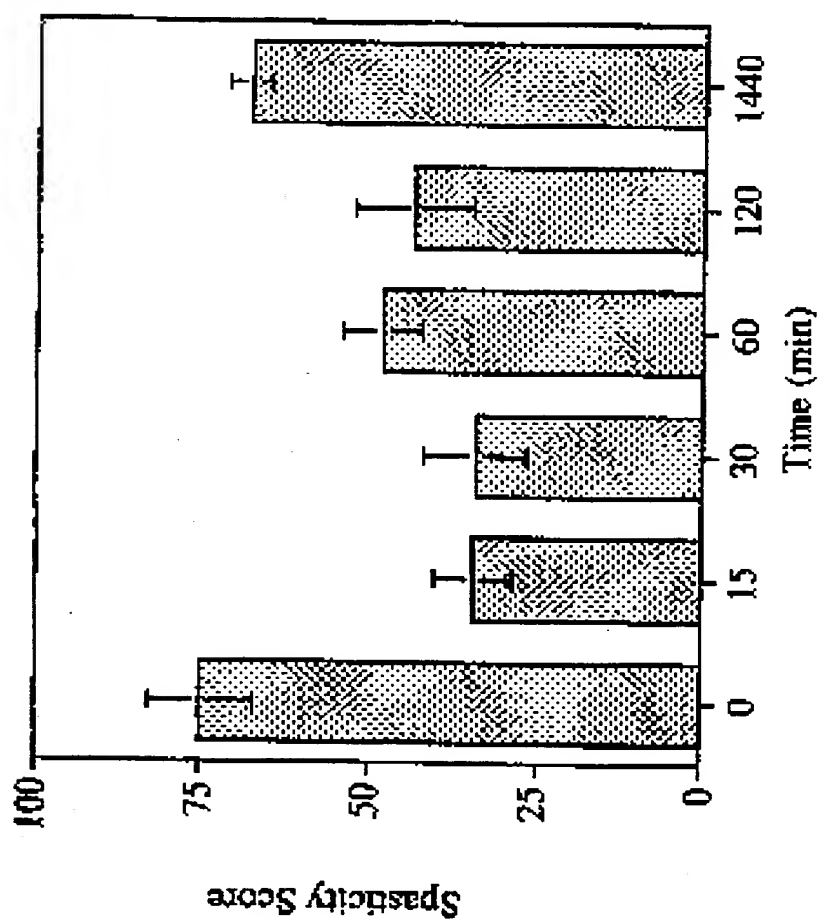


Figure 2. Isovaleramide (300 mg/kg, i.p.) reduces the spasticity scores in chronic spinalized rats.

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The Flexor Reflex in the Chronic Spinalized Rat

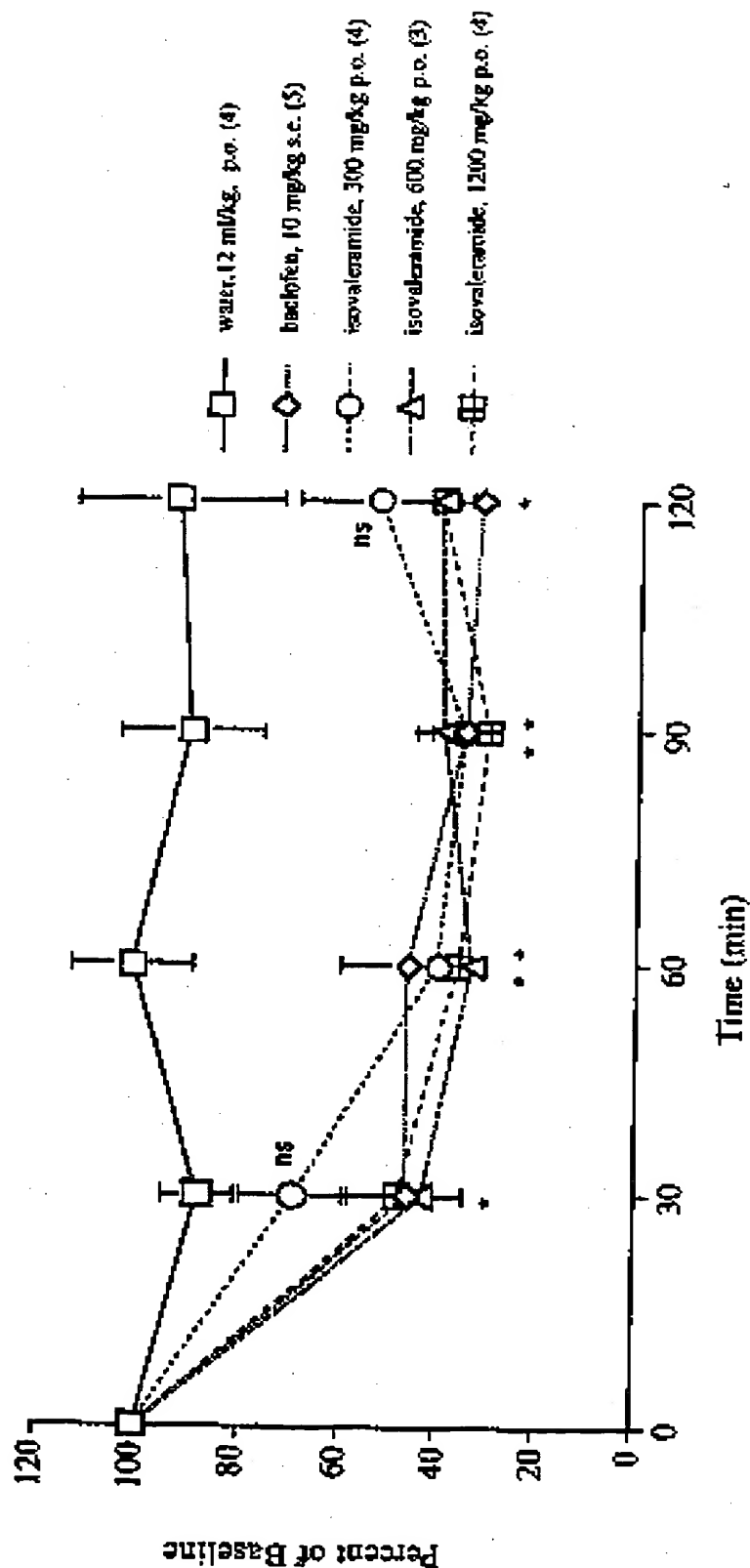


Figure 3. Isovaleramide produces a dose- and time-dependent reduction in the magnitude of the flexor reflex in chronic spinalized rats. * $p < 0.05$, ** $p < 0.01$, NS = not significant (1-way ANOVA and post-hoc Dunnett's t-test).

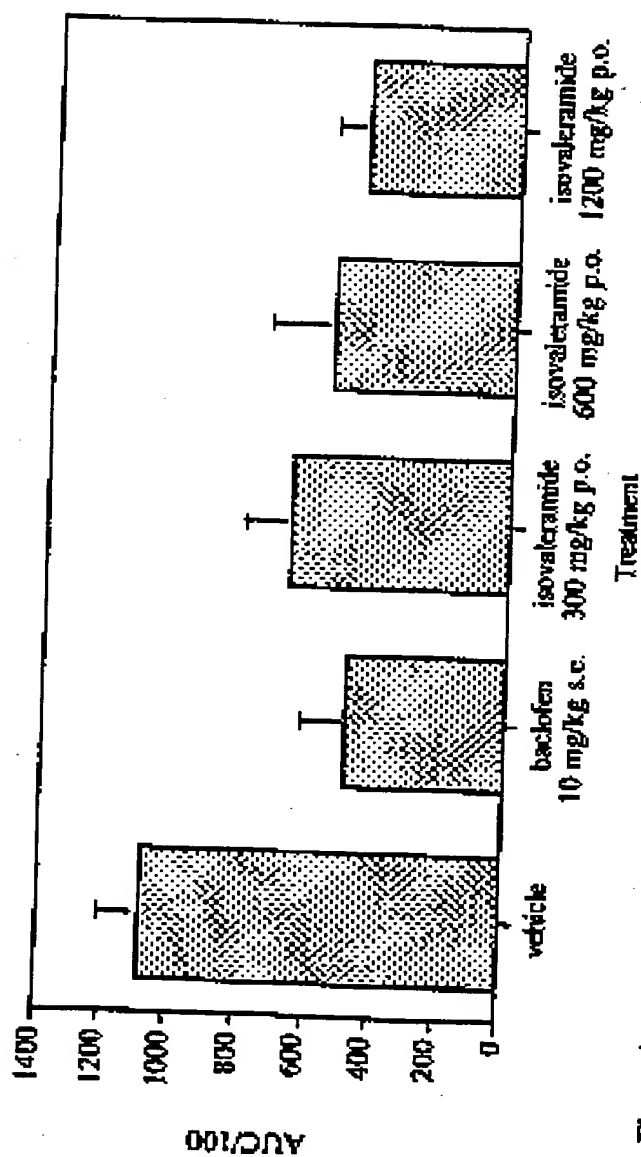


Figure 4. Isovaleramide and baclofen produce a similar reduction of the flexor reflex in chronic spinalized rats. The responses from Figure 3 are converted to a total area-under-the-curve for the 2 hour measurement. All drug-treated groups are significantly different from the vehicle-treated group ($p < 0.05$, One Way Analysis of Variance).